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(71) Applicant: JOHNSON & JOHNSON CONSUMER COMPANIES, INC. [–/US]; Grandview Road, Skillman, NJ 08558 (US).

(72) Inventors: SHAPIRO, Stanley, S.; 10 Plymouth Drive, Livingston, NJ 07039 (US). NIEMEC, Susan; 1808 Waterford Road, Yardley, PA 19057 (US). KUNG, John; 303 Classon Court, Somerset, NJ 08873 (US). SEIBERG, Miri; 168 Herrontown Road, Princeton, NJ 08540 (US).

(74) Agents: CIAMPORCERO, Audley, A. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933–7003 (US).

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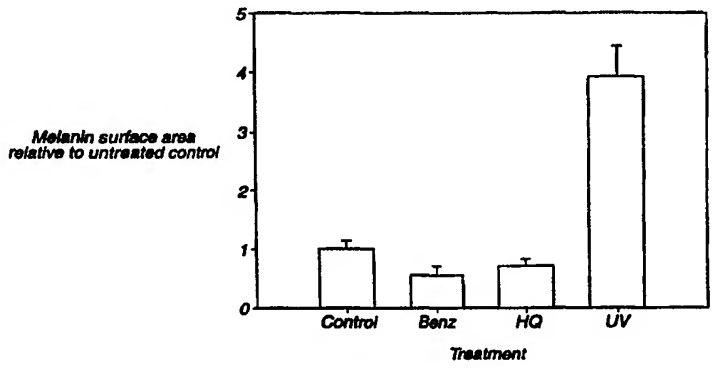
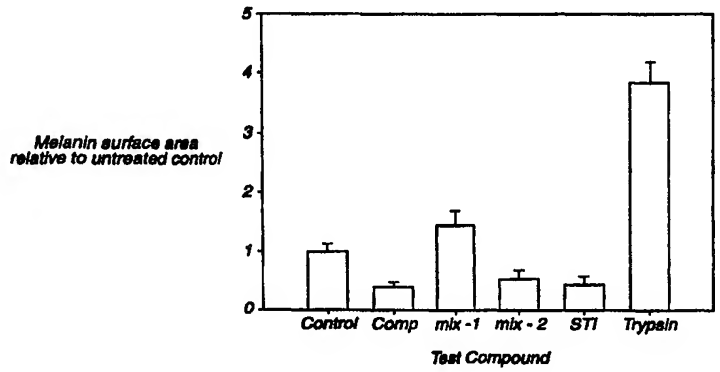
(54) Title: METHODS FOR TREATING SKIN PIGMENTATION

(57) Abstract

This invention relates to methods and compositions for bringing about changes in skin pigmentation. More particularly, this invention relates to compounds which affect melanogenesis and can used as depigmenting agents or as agents for darkening skin utilizing the PAR–2 pathway.





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METHODS FOR TREATING SKIN PIGMENTATION

1. Field of the Invention

This invention is related to methods and compositions for bringing about skin pigmentation and/or for causing skin depigmentation. More particularly, this invention relates to compounds which affect melanogenesis and can be used as depigmenting agents or as agents for darkening skin.

2. Background of the Invention

Skin coloring has been of concern to human beings for many years. In particular, the ability to remove hyperpigmentation, such as found in age spots, freckles or aging skin generally, is of interest to individuals desiring a uniform complexion. In certain areas of the world, general body whitening is desirable. There are also hypopigmentation and hyperpigmentation disorders that are desirable to treat. Likewise, the ability to generate a tanned appearance without incurring photodamage due to solar radiation is important to many individuals. There have been many methods proposed to accomplish depigmentation, as well as to accomplish darkening of the skin. For example, kojic acid, hydroquinone, retinoids and other chemical compounds have been used for depigmentation. Dihydroxyacetone and like chemical compounds have been utilized for their ability to "tan" the skin without exposure to the sun.

Many of these previous solutions have not been found acceptable. There is often a distinct line of demarcation between the areas of skin to which such previous compositions have been applied. Therefore,

precise application of all these compounds is necessary in order to achieve the desired result. Many of these compounds have been found to be quite irritating to the skin and therefore undesirable for use.

The understanding of the chemical and enzymatic basis of melanogenesis is heavily documented. Melanocytes migrate from the embryonal neural crest into the skin to produce secretory granules, melanosomes, which produce melanin. Melanogenesis occurs within the melanosome, and the melanin is later distributed to keratinocytes via the melanocyte dendrites. The key enzyme in melanogenesis is tyrosinase, which initiates a cascade of reactions which convert tyrosine to the biopolymer melanin. Two tyrosinase-related proteins (TRP's) are known, TRP-1 and TRP-2. These proteins share with tyrosinase about 40% homology and have catalytic activities as well as regulatory roles in melanogenesis. TRP-1 is the most abundant glycoprotein in melanocytes.

In spite of the fact that the chemical and enzymatic basis of melanogenesis is well-documented, its regulation at the cellular level is only partially understood. Tyrosinase and the TRP's share structural and biological properties with the lysosomal-associated membrane protein (LAMP) gene family, therefore their targeting to the melanosomal membrane might induce their activation. A phosphorylation/dephosphorylation reaction at the cytoplasmic tails of these proteins could be involved in the regulation of melanogenesis. The beta isoform of the Protein Kinase C (PKC) family has been shown to regulate human melonogenesis through tyrosinase activation. Gene expression of tyrosinase, TRP-1 and TRP-2 is coordinated. All three enyzmes are

expressed in human epidermis. In melanocytes co-cultured with keratinocytes, these transcripts are expressed at a ratio of 45:45:10, respectively. In melanocytes cultured alone, only TRP-1 transcripts are present, indicating that a keratinocyte-derived signal is involved in the coordinate expression of these genes. The regulation of keratinocyte-melanocyte interactions and the mechanism of melanosome transfer into keratinocytes are not yet understood.

The Protease-activated receptor-2 (PAR-2) is a seven transmembrane G-protein-coupled receptor, that is related to, but distinct from the thrombin receptors (TR, also named PAR-1, and PAR-3) in its sequence. Both receptors are activated proteolytically by an arginine-serine cleavage at the extracellular domain. The newly created N-termini then activate these receptors as tethered ligands. Both receptors could be activated by trypsin, but only the TRs are activated by thrombin. Only PAR-2 is activated by mast cell tryptase. Both receptors could also be activated by the peptides that correspond to their new N-termini, independent of receptor cleavage. SLIGRL, the mouse PAR-2 activating peptide, is equipotent in the activation of the human receptor. While the function of the TR is well documented, the biology of the PAR-2 has not yet been fully identified. A role for PAR-2 activation in the inhibition of keratinocyte growth and differentiation has been recently described (Derian et al., "Differential Regulation of Human Keratinocyte Growth and Differentiation by a Novel Family of Protease-activate Receptors", *Cell Growth & Differentiation*, Vol. 8, pp. 743-749, July 1997).

Summary of the Invention

In accordance with this invention, we have found a method for affecting changes in mammalian skin pigmentation comprising topically applying to the skin of a mammal a compound which affects the PAR-2 pathway. The compositions of this invention may contain one or more compounds that act as trypsin, as tryptase, as serine protease or as PAR-2 agonists, for increase in pigmentation. Alternatively, they may contain one or more compounds that act as serine protease inhibitors, trypsin inhibitors, thrombin inhibitors, tryptase inhibitors, as PAR-2 pathway inhibitors or as a PAR-2 antagonist for decrease in pigmentation, or "depigmentation".

As used herein, "mammal" means any member "of the higher vertebrate animals comprising the class "Mammalia", as defined in Webster's Medical Desk Dictionary 407 (1986), and includes but is not limited to humans. As used herein, "receptor" shall include both intracellullar and extracellular receptors and shall mean those molecules capable of receiving and transducing a signal. The term PAR-2 refers to the protease-activated receptor-2 or a related protease activated receptor. The Protease-activated receptor-2 (hereinafter, "PAR-2") is a serine-protease activated receptor that is expressed in numerous tissues, including keratinocytes and fibroblasts. The thrombin receptor (also named PAR-1, hereinafter, "TR") is a serine-protease activated receptor that is expressed in numerous tissues, including keratinocytes. The biological roles of PAR-2 and TR in skin are not entirely known. However, we have found that interactions between keratinocytes and melanocytes, via

the PAR-2 pathway, affect melanogenesis. We have found that thrombin inhibitors, and/or tryptase inhibitors, and/or trypsin inhibitors and PAR□□ antagonists can be used as depigmenting agents without irritation of the skin. PAR-2 agonists and serine proteases such as trypsin and tryptase can be used as darkening agents. Furthermore, PAR-2 could be useful as a target for whitening and darkening agents.

Brief Description of the Drawings

Fig. 1A is a graph depicting the increase or decrease in relative pigmentation of epidermal equivalents containing melanocytes treated with known pigmenting and depigmenting agents in accordance with the methods of this invention.

Fig. 1B is a graph depicting the increase or decrease in relative pigmentation in epidermal equivalents containing melanocytes treated in accordance with the methods and compositions of this invention.

Fig. 2 is a group of images of epidermal equivalents containing melanocytes treated with PAR-2 agonists and Compound I.

Fig. 3 is a graph depicting the increase or decrease in relative pigmentation in epidermal equivalents containing melanocytes treated in accordance with the methods and compositions of this invention.

Fig. 4A is a graph depicting the dose/response with respect to pigmentation in epidermal equivalents containing melanocytes when treated with compositions of this invention.

Fig. 4B is a graph depicting the response of epidermal equivalents containing melanocytes after

exposure to ultraviolet light followed by treatment with compositions of this invention.

Fig. 5A is a photograph depicting gels showing the expression of TR and PAR-2 in skin, melanoma cells and epidermal equivalents containing melanocytes.

Fig. 5B is a photograph depicting gels showing the expression of TR and PAR-2 by primary human melanocytes.

Fig. 6A and 6B are photographs depicting gels showing the expression of various genes after treatment with different concentrations of Compound I and SLIGRL.

Fig. 7 is a graph showing the effects of different compositions of this invention on the brightness of guinea pig nipple pigmentation.

Fig. 8 is a photograph of Yucatan Swine skin which has been treated with compositions of this invention for depigmentation of skin.

Fig. 9 is a graph depicting the brightness of Yucatan Swine skin during the course of treatment in accordance with the methods and compositions of this invention.

Fig. 10A, 10B, 10C and 10D are photographs of F&M stained histological sections of Yucatan Swine skin treated with compositions containing Compound I in accordance with methods of this invention at concentrations of 0, 10 μM, 50 μM and 250 μM respectively.

Figs. 11A, 11B and 11C are photographs of electron micrographic views of epidermal equivalents containing melanocytes treated with compositions of this invention.

Figs. 11E, 11F and 11H are photographs of electron micrographic views of Yucatan Swine skin treated with compositions of this invention.

Figs. 11D and 11G are photographs of electron micrographic views of untreated sites of Yucatan Swine skin.

Figs. 12A, 12B, 12C, 12D and 12E are photographs of histological F&M stained sections of Yucatan Swine skin, as follows: 12A shows untreated skin; 12B shows skin treated with compositions of this invention after eight weeks of treatment; 12C shows skin one week after stopping treatment; 12D shows skin two weeks after stopping treatment and 12E shows skin four weeks after stopping treatment.

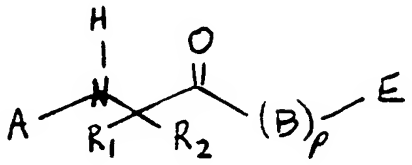
Fig. 13 is a photograph of F&M stained histological sections taken from Yucatan Swine skin treated with compositions of this invention.

Fig. 14 contains ultraviolet and visible light digital photographs of human skin prior to treatment and subsequent to treatment with compositions of this invention.

Detailed Description of the Preferred Embodiments

We have discovered that trypsin, tryptase and PAR-2 agonists can be used to increase pigmentation and that trypsin inhibitors, and/or tryptase inhibitors, and/or thrombin inhibitors and PAR-2 antagonists act to decrease pigmentation in mammalian skin. In our opinion, some of the compounds described in U.S. Patent No. 5,523,308, which is hereby incorporated herein by reference, and behave as thrombin and/or trypsin and/or tryptase inhibitors, will be useful in methods of this invention. Some of these compounds are also described in Costanzo, et al., "Potent Thrombin Inhibitors That Probe the S_1' Subsite: Tripeptide Transition State Analogues Based on a Heterocycle-Activated Carbonyl

Group", J. Med. Chem., **1996**, Vol. 39, pp. 3039-3043 and have the following structural formula:



wherein:

A is selected from the group consisting of C_{1-8}alkyl, carboxyC_{1-4}alkyl, C_{1-4}alkoxycarbonylC_{1-4}alkyl, phenylC_{1-4}alkyl, substituted phenylC_{1-4}alkyl (where the phenyl substituents are independently selected from one or more of, C_{1-4} alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4} alkoxycarbonyl), formyl, C_{1-4}alkoxycarbonyl, C_{1-2}alkylcarbonyl, phenylC_{1-4}alkoxycarbonyl, C3-7cycloakylcarbonyl, phenylcarbonyl, substituted phenylcarbonyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4} alkoxycarbonyl), C_{1-4}alkylsulfonyl, C_{1-4}alkoxysulfonyl, perfluoroC_{1-4}alkyl-sulfonyl, phenylsulfonyl, substituted phenylsulfonyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4} alkoxycarbonyl), 10-camphorsulfonyl, phenylC_{1-4}alkysulfonyl, substituted phenylC_{1-4}alkysulfonyl, C_{1-4}alkylsulfinyl, perfluroC_{1-}

$_4$alkylsulfinyl, phenylsulfinyl, substituted phenylsulfinyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), phenylC_{1-4}alkylsulfinyl, substituted pehnylC_{1-4}alkylsulfinyl, 1-naphthylsulfonyl, 2-naphthylsulfonyl or substituted naphthylsulfonyl (where the naphthyl substituents are independently selected from one or more of, C_{1-4}alkyl,perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, carboxy or C_{1-4}alkoxyy-carbonyl), 1-naphthylsulfinyl, 2-naphthylsulfinyl or substituted naphthylsulfinyl (where the naphthyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl);

a D or L amino acid which is coupled as its carboxy terminus to the nitrogen depicted in formula I and is selected from the group consisting of alanine, asparagine, 2-azetidinecarboxylic acid, glycine, N-C_{1-8}alkyglycine, proline, 1-amino-1-cycloC_{3-8}alkylcarboxylic acid, thiazzolidine-4-carboxylic acid, 5,5-dimethylthiazolidine-4-carboxylic acid, oxadolidine-4-carboxylic acid, pipecolinic acid, valine, methionine, cysteine, serine, threonine, norleucine, leucine, tert-leucine, isoleucine, phenylalanine, 1-naphthalanine, 2-naphthalamine, 2-thienylalanine, 3-thienylalanine, [1,2,3,4]-tetrahydroisoquinoline- 1-carboxylic acid and 1,2,3,4,]-tetrahydroisoquinoline-2-caroboxylic acid

where the amino terminus of said amino acid is connected to a member selected from the group consisting of C_{1-4}alkyl,tetrazol-5yl-C_{1-2}alkyl, carboxytC_{1-4}alkyl, C_{1-4}alkoxycarbonylC14alkyl, phenylC_{1-4}alkyl, substituted phenyl C_{1-4} alkyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboy or C_{1-4}alkoxycarbonyl), 1,1-diphenylC_{1-4}alkyl, 3-phenyl-2-hydroxypropionyl, 2,2-diphenyl- 1-hydroxyethylcarbonyl, [1,2,3,4]-tetrahydroisoquinoline- 1-carbonyl, [1,2,3,4]-tetrahydroisoquinoline-3,carbonyl, 1-methylamino-1-cyclohexanecarbonyl, 1-hydroxy-1-cyclohexanecarbonyl, 1-hydroxy-1-pheny-lacetyl, 1-cyclohexyl-1-hydroxyacetyl, 3-phenyl-2-hydroxypropionyl, 3,3-diphenyl-2-hydroxypropionyl, 3-cyclohexyl-2-hydroxypropionyl, formyl, C_{1-4}alkoxycarbonyl,C_{1-12}alkylcarbonyl, perfluoroC_{1-4}alkyl, C_{1-4}alkylcarbonyl, phenylC_{1-4}alkylcarbon1, substituted phenylC_{1-4}alkylcarbonyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo amido, nitro amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl) 1,1-diphenylC_{1-4}alkylcarbonyl, substituted 1,1-diphenylC_{1-4}alkylcarbonyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoro C_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4} alkoxy-carbonyl), perfluoroC_{1-4}alkysulfonyl, C_{1-4}alkysulfonyl, C_{1-4}alkoxysulfonyl, phenysulfonyl, substituted phenylsulfonyl (where the phenyl substituents are independently selected from one or

more of, C-1alkyl, perfluoro C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), 10-cxamphorsulfonyl, phenylC_{1-4}alkylsulfonyl, substituted phenylC_{1-4}alkylsufonyl, perfluroC_{1-4}alkysulfinyl, C-14alkysulfinyl, phenylsulfinyl, substituted phenysulfinyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoro C_{1-4}alkyl, C_{1-4} alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4} alkylamino, C_{1-4} dialkylamino, carboxy or C_{1-4} alkoxycarbonyl), 1-naphthysulfonl, 2-naphthylsulfonyl, substituted naphthylsulfonyl (where the naphthyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboy or C_{1-4}alkoxycarbonyl),1-naphthysulfinyl, 2-naphthysulfinyl, and substituted naphthylsulfinyl (where the naphthyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo amido, nitro, amino, C_{1-4}alkylamino, C104dialkylamono, carboxy or C-14alkoxycarbonyl):
or a poly peptide comprised of two amino acids, where the first amino acid is a D or L amino acid, bound via its carboxy terminus tot he nitrogen depicted in Formula I and is selected from the group consisting of glycine, N-C_{1-8}alkylglycine, alanine, 2-azetidinecarboxylic acid, proline, thiazolidine-4-carboxylic acid, 5.5-dimethylthiazolidine-4-carboxylic acid, oxazolidine-4-carboxylic acid, 1-amino-1-cycloC_{3-8} alkylcarboxylic acid, 3-hydroxypropoline, 4-hydroxyproline, 3-(C_{1-4}alkoxy)proline,

4(C_{1-4}alkoxy)proline, 3,4-dehydroprline, 2,2-dimethyl-4-thiazolidine carboxylic acid, 2.2-dimethyl-4-oxadolidine carboxylic acid, pipecolinic acid, valine, methionine, cysteine, asparagine, serine, threonine, leucine, tert-leucine, isoleucine, phenylalanine, 1-naphthalanine, 2-naphthalanine, 2-thienylalanine, 3-thienylalnine, [1,2,3,4]-tetrahydroisoquinoline- 2-carboxylic acid, aspartic acid-4-C_{1-4}alkyl ester and glutamic acid 5-C_{1-4}alkyl ester

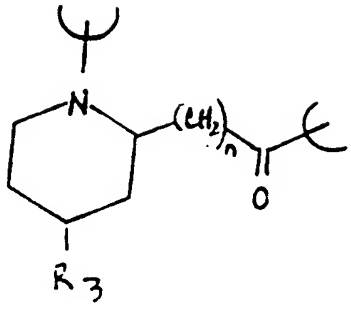
and the second D or L amino acid, is bound to the amino terminus of said first amino acid, and is selected from the group consisting of phenylalanine, 4-benzolyphenylalanine, 4-carboxyphenylalanine, 4-(Carboxy C1-2alkyl)phenylalanine, substituted phenylalanine (where the phenyl substituents are independently selected from one or more of C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), 3-benzothienylalanine, 4-biphenylalanine, homophenylalanine, octahydroindole-2-carboxylic acid, 2-pyridylalanine, 3-pyridylalanine, 4-thiazolyalanine, 2-thienylalanie, 3-(3-benzothienyl)alanine, 3-thienylalanine, tryptophan, tyrosine, asparagine, 3-tri-C_{1-4}alkylsilylalanine, cyclohexylglycine, diphenylglycine, phenylglycine, methionine sulfoxide, methionine sulfone, 2,2-dicyclohexylalanine, 2-(1-naphthylalanine), 2-(2-naphthylalanine), phenyl substituted phenylalanine (where the substituents are selected from C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4} alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialylamino, carboxy or C_{1-4} alkoxycarbonyl), aspartic acid, aspartic acid-4C_{1-4}alkyl, perfluoroc_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-}

$_4$alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbony), aspartic acid, aspartic acid-4-C_{1-4}alkyl ester glutamic acid, glutamic acid-5-C_{1-4} alkyl ester, cycloC3-salkylaalanine, substituted cycloC_{3-8}alkylalanine (where the ring substituents are carboxy, C_{1-4} alkyl ester, cycloC3-salkylalanine, substituted cycloC_{3-8}alkylalanine (where the ring substituents are carboxy, C_{1-4}alkylcarboxy, C_{1-4}alkoxycarbonyl or aminocarbonyl), 2,2-diphenylalanine and all alpha-C_{1-5}alkyl of all amino acid derivatives thereof, where the amino terminus of said second amino acid is unsubstituted or monosubstituted with a member of the group consisting of formyl, C1-12 alkyl, tetrazol-5-ylC1-2alkyl, carboxyC1-8 alkyl, carboalkoxyC_{1-4}alkyl, phenyl C_{1-4}alkyl, substituted phenylC_{1-4}alkyl (where the phenyl substituents or independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), 1,1-dipehnylC_{1-4}alkyl, C1-6alkoxycarbonyl, phenylC1-6alkoxycarbonyl, C1-2alkylcarbonyl, perfluoroC_{1-4}alkylCo-4alkylcarbonyl, phenyC_{1-4}alkylcarbonyl, substituted phenyC_{1-4}alkylcarbonyl(where the phenyl substituents are independently selected from one or more of C_{1-4}alkyl, perfluoro C_{1-4}alkyl, C_{1-4} alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), 1,1-diphenylC_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxycarbonyl), 10-camphorsulfonyl, phenylC_{1-4}alkysulfonyl, substituted phenylC_{1-4}alkylsulfonyl, C_{1-4}alkysulfinyl, perfluoro C_{1-4}alkylsulfinyl, phenylsulfinyl, substituted phenylsulfinyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy,

hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamono, C_{1-4}dialkylamono, carboxy or C_{1-4}alkoxycarbonyl), phenylC_{1-4}alkylsulfinyl, substituted phenylC_{1-4}alkylsulfinyl 1-naphthylsulfonyl, 2-naphthylsulfonyl, substituted naphthylsulfonyl (where the naphthyl substituent is selected from C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), 1-haphthyl-sulfinyl, 2-haphthylsulfinyl and substituted naphthyl-sulfinyl (where the naphthyl substituent is selected from C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C-14dialkylamino, carboxy or C_{1-4}alkoxycarbonyl); R_1 is selected from the group consisting of hydrogen and alkyl; R_2 is selected from the group consisting of aminoC2-salkyl, guanidinoC_{2-5}alkyl, C_{1-4}alkylguanidinoC_{2-5}alkyl,diC_{1-4}alkylguanidinoC_{2-5}alkyl, amidinoC_{2-5}alkyl, C_{1-4}alky-lamidinoC_{2-5}alkyl, diC_{1-4}alky-lamidinoC_{2-5}alkyl, C_{1-3}alkoxyC_{2-5}alkyl, phenyl, substituted phenyl (where the substituents are independently selected from one or more of amino, amidino, guanidino, C_{1-4}alkylamino, C_{1-4}dialkylamino, halogen, perfluoro C_{1-4}alkyl, C_{1-4}alkyl, C_{1-3} alkoxy or nitro), benzyl, phenyl substituted benzyl (where the substituents are independently selected from one or more of, amino, amidino, guanidino, C_{1-4}alkylamino, C_{1-4}dialky-lamino, halogen, perfluoro C_{1-4}alkyl, C1-04alkyl, C_{1-3}alkoxy or nitro), hydroxyC_{2-5}alkl, C`$_{1-5}$alkylaminoC_{2-5}alkyl, C_{1-5}dialkylaminoC_{2-5}alkyl, 4-aminocyclohexylC_{0-2}alkyl and C_{1-5}alkyl;

p is 0 or 1;

B is



where n is 0-3, R_3 is H or C1-5alkyl and the carbonyl moiety of B is bound to E;

E is a heterocycle selected from the group consisting of oxazolin-2-yl, oxazol-2-yl, thiazol-2-yl, thiazol-5-yl, thiazol-4-yl, thiazolin-2-yl, imidazol-2-yl, 4-oxo-2-quinoxalin-2yl, 2-pyridyl, 3-pyridyl, benzo[b}thiophen-2-yl, triazol-4-yl triazol-6-yl, pyrazol-2-yl, 4,5,6,7-tetrahydrobenzothiazol-2yl, naphtho[2,1-d]thiazol-2-yl, naphtho[1-2-d]thiazol-2-yl quinoxalin- 2-yl, isoquinolin-1-yl, isoquinolin-3-yl, benzo [b]furan-2-yl, [pyrazin-2-yl, quinazolin-2-yl, isothiazol-5-yl, isothiazol-3-yl, purin-8yul and a substituted heterocycle where the substituents are selected from C_{1-4} from C-14alky, perfluoro C_{1-4}alkyl,C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy, C_{1-4}alkoxycarbonyl, hydroxy or phenylC_{1-4} alkylaminocarbonyl;

or pharmaceutically acceptable salts thereof.

More particularly, in our opinion, some of the compounds of the foregoing formula containing a d-phenylalanine-proline-arginine motif should be effective in inhibiting the PAR-2 pathway and causing

depigmentation. One particularly preferred compound which acts as a thrombin and trypsin inhibitor and is active in depigmenting mammalian skin is (S)-N-Methyl-D-phenylalanyl-N-[4-[(aminoiminomethyl)amino]-1-(2-benzothiazolylcarbonyl)butyl]-L-prolinamide (Chemical Abstracts name) (hereinafter referred to as "Compound I"). We suggest that other compounds which are analogs or function similarly to Compound I and are set forth in U.S. Patent No. 5,523,308 may be active in the methods and compositions of this invention. Other compounds that inhibit trypsin, such as serine protease inhibitors, and in particular, soybean trypsin inhibitor (STI) will also be useful in methods of this invention. Soybean, limabean and blackbean extracts, and other natural products made from these beans, such as, but not limited to, bean milk, bean paste, miso and the like, also serve to reduce pigmentation by this mechanism.

Additional sources of serine protease inhibitors may be extracted from the species belonging to the following plant families: Solanaceae (e.g., potato, tomato, tomatilla, and the like); Gramineae (e.g., rice, buckwheat, sorghum, wheat, barley, oats and the like); Cucurbitaceae (e.g., cucumbers, squash, gourd, luffa and the like); and, preferably, Leguminosae (e.g., beans, peas, lentils, peanuts, and the like).

While not willing to be bound by the following theory, we theorize that the compounds capable of affecting the pigmentation of the skin do so by interacting directly or indirectly with the keratinocyte PAR-2 or with its activating protease, and thereby affect melanogenesis, directly or indirectly. Possibly, the compounds of this invention induce, in the case of increased pigmentation or reduce, in the case of decreased pigmentation, the signal to transport

melanosomes by melanocytes, or to receive melanosomes by keratinocytes in the skin.

The compounds which are active in the compositions and methods of this invention may be delivered topically by any means known to those of skill in the art. If the delivery parameters of the topically active pharmaceutical or cosmetic agent so require, the topically active composition of this invention may preferably be further composed of a pharmaceutically or cosmetically acceptable vehicle capable of functioning as a delivery system to enable the penetration of the topically active agent into the skin.

One acceptable vehicle for topical delivery of some of the compositions of this invention, particularly proteins such as trypsin and STI, may contain liposomes. The liposomes are more preferably non-ionic and contain a) glycerol dilaurate (preferably in an amount of between about 5% and about 70% by weight); b) compounds having the steroid backbone found in cholesterol (preferably in an amount of between about 5% and about 45% by weight); and c) one or more fatty acid ethers having from about 12 to about 18 carbon atoms (preferably in an amount of between about 5% and about 70% by weight collectively), wherein the constituent compounds of the liposomes are preferably in a ratio of about 37.5:12.5:33.3:16.7. Liposomes comprised of glycerol dilaurate / cholesterol/ polyoxyethylene -10-stearyl ether/polyoxyethylene-9-lauryl ether (GDL liposomes) are most preferred. Preferably the liposomes are present in an amount, based upon the total volume of the composition, of from about 10 mg/mL to about 100 mg/mL, and more preferably from about 20 mg/mL to about 50 mg/mL. A ratio of about 37.5:12.5:33.3:16.7 is most

preferred. Suitable liposomes may preferably be prepared in accordance with the protocol set forth in Example 1, though other methods commonly used in the art are also acceptable. The above described composition may be prepared by combining the desired components in a suitable container and mixing them under ambient conditions in any conventional high shear mixing means well known in the art for non-ionic liposomes preparations, such as those disclosed in Niemiec *et al.*, "Influence of Nonionic Liposomal Composition On Topical Delivery of Peptide Drugs Into Pilosebacious Units: An In Vivo Study Using the Hamster Ear Model," 12 Pharm. Res. 1184-88 (1995) ("Niemiec"), which is incorporated by reference herein in its entirety. We have found that the presence of these liposomes in the compositions of this invention may enhance the depigmenting capabilities of some of the compositions of this invention.

Other preferable formulations may contain, for example, soybean milk or other liquid formulations derived directly from legumes or other suitable plant. For example, such a formulation may contain a large proportion of soybean milk, an emulsifier that maintains the physical stability of the soybean milk, and, optionally a chelating agent, preservatives, emollients, humectants and/or thickeners or gelling agents.

Oil-in-water emulsions, water-in-oil emulsions, solvent-based formulations and aqueous gels known to those of skill in the art may also be utilized as vehicles for the delivery of the compositions of this invention.

The source of active compound to be formulated will generally depend upon the particular form of the compound. Small organic molecules and peptidyl

fragments can be chemically synthesized and provided in a pure form suitable for pharmaceutical/cosmetic usage. Products of natural extracts can be purified according to techniques known in the art. Recombinant sources of compounds are also available to those of ordinary skill in the art.

In alternative embodiments, the topically active pharmaceutical or cosmetic composition may be optionally combined with other ingredients such as moisturizers, cosmetic adjuvants, anti-oxidants, bleaching agents, tyrosinase inhibitors and other known depigmentation agents, surfactants, foaming agents, conditioners, humectants, fragrances, viscosifiers, buffering agents, preservatives, sunscreens and the like. The compositions of this invention may also contain active amounts of retinoids (i.e., compounds that bind to any members of the family of retinoid receptors), including, for example, tretinoin, retinol, esters of tretinoin and/or retinol and the like.

The topically active pharmaceutical or cosmetic composition should be applied in an amount effective to affect changes in the pigmentation of mammalian skin. As used herein "amount effective" shall mean an amount sufficient to cover the region of skin surface where a change in pigmentation is desired. Preferably, the composition is liberally applied to the skin surface such that, based upon a square cm of skin surface, from about 2 μl /cm^2 to about 200 μl /cm^2 of topically active agent is present when a change in pigmentation is desired. When using a thrombin and trypsin inhibitor such as Compound I or its analogs, whether synthetically- or naturally-derived in a formulation, such an active compound should be present in the amount of from about 0.0001% to about 15% by weight/volume of

the composition. More preferably, it should be present in an amount from about 0.0005% to about 5% of the composition; most preferably, it should be present in an amount of from about 0.001 to about 1% of the composition. Of course, these ranges are suggested for the foregoing components. The lower set of ranges is intended to be efficacious for PAR-2 pathway agonists/antagonists and/or inhibitors having high therapeutic indices and which do not require significantly larger concentrations or doses to be effective in the methods of this invention. Such compounds may be synthetically- or naturally-derived.

Liquid derivatives and natural extracts made directly from plants or botanical sources may be employed in the compositions of this invention in a concentration (w/v) from about 1 to about 99%. Fractions of natural extracts and naturally-derived protease inhibitors such as STI may have a different preferred range, from about 0.01% to about 20% and, more preferably, from about 1% to about 10% of the composition. Of course, mixtures of the active agents of this invention may be combined and used together in the same formulation, or in serial applications of different formulations.

We have unexpectedly found that when topically active agents, such as PAR-2 agonists and/or inhibitors and trypsin and/or thrombin and/or tryptase and/or their inhibitors, are topically applied to an animal's skin, a significant change in pigmentation was achieved. Preferably, depigmenting agents (as well as other pigmentation-affecting agents of this invention) are applied to the skin of a mammal at a relatively high concentration and dose (from about 0.005% to about 1% for compounds having high therapeutic indices such

as Compound I and related compounds; from about 20% to about 99% for liquid derivatives and extracts of botanical materials; and from about 1% to about 20% for fractions of natural extracts and naturally-derived protease inhibitors such as STI or mixtures thereof) between one and two times daily for a period of time until the skin evidences a change in pigmentation. This may be for from about four to about ten weeks or more. Thereafter, once the change in pigmentation has been achieved, a lower concentration and dose (from about 0.00001% to about 0.005% for compounds having high therapeutic indices such as Compound I and related compounds; from about 10% to about 90% for liquid derivatives and extracts of botanical materials; and from about 0.01% to about 5% for fractions of natural extracts and naturally-derived protease inhibitors such as STI or mixtures thereof), of active ingredient may be applied on a less frequent time schedule, e.g., about once per day to about twice per week. The effects of the active agents of this invention are reversible, therefore, in order to maintain these effects, continuous application or administration should be performed. The invention illustratively disclosed herein suitably may be practiced in the absence of any component, ingredient, or step which is not specifically disclosed herein.

Several examples are set forth below to further illustrate the nature of the invention and the manner of carrying it out, but do not serve to limit the scope of the methods and compositions of this invention.

Example 1: Protease Inhibitors Affect Pigmentation

In order to study the possible roles of the PAR-2 pathway in pigmentation, an *in vitro* epidermal

equivalent system was used. The epidermal equivalent system used contained melanocytes. One epidermal equivalent system which is useful in performing this study is the MelanoDerm system, available commercially from MatTek Co. This system contains human normal melanocytes, together with normal, human-derived epidermal keratinocytes, which have been cultured to form a multi-layered, highly differentiated model of the human epidermis. In the following examples, equivalents were treated with test compounds for three days and samples were harvested on the fourth day after beginning of treatment. The harvested equivalents were stained with DOPA (a substrate for tyrosinase) and H&E (a standard histological stain) or with Fontana-Mason (F&M) staining, another stain known to those of skill in the art. F&M staining is a silver staining technique that clearly and cleanly marks melanins which have high silver nitrate reducing activity. Multilayered human epidermal equivalents containing melanocytes were used as an *in vitro* model system to study the effect of protease inhibitors on melanogenesis. Epidermal equivalents used were commercially available as MelanoDerm from MatTek of Ashland, MA. These equivalents are known to respond to ultraviolet B ("UVB") irradiation and known whitening agents such as benzaldehyde and hydroquinone by increasing and reducing pigmentation, respectively. The MelanoDerm epidermal equivalents were exposed to benzaldehyde (available from Sigma of St. Louis, MO), hydroquinone (available from Sigma) and UVB irradiation. UV irradiation was performed with a UVB FS light source in an exposure chamber, with plate covers removed and Phosphate-buffered saline (PBS, from Gibco-BRL, Gaithersburg, MD) present in the lower chamber. UVB intensity was

measured with a UVX radiometer (UVP Inc., San Gabriel, CA). Equivalents were treated with 0.1-0.12 J/cm^2 . No loss of viability was observed in equivalents treated with up to 0.3 J/cm^2 .

On the fourth day of exposure to the test compounds/ultraviolet irradiation, the equivalents were fixed, sectioned and stained, or stained as whole without sectioning. MelanoDerm equivalents were formalin fixed and put in paraffin blocks, and sections from the MelanoDerm equivalents were stained in accordance with the following standard procedures: (1) H&E, (2) DOPA + H&E and (3) Fontana-Mason ("F&M") using standard techniques known to those of skill in the art. Alternatively, whole MelanoDerm equivalents were stained and their images were captured for image analysis. At least three sections per equivalent, three equivalents per experiment were processed. Each experiment was repeated three time. DOPA is a substrate for tyrosinase. F&M identifies silver nitrate reducing molecules, which identifies primarily melanins. F&M stained sections were used for image analysis using Optomax Image Analysis Systems, from Optomax Inc., Hollis, NH. Alternatively, Empire Images database 1.1 was used on a Gateway 2000 P5-100 computer (Media Cybernetics, Silver Springs, MD) for capturing images. Image Pro Plus version 4.0 was used for image analysis. Parameters measured were as follows: (1) level of pigmentation within individual melanocytes and (2) number of pigmented melanocytes per field, for the Optomax system, or (1) the surface area of silver deposits within melanocytes and (2) the number of pigmented melanocytes for the Image Pro system. Using the Optomax system, surface area of silver deposits within individual melanocytes was measured in 60

melanocytes, using multiple sections from triplicate equivalents per treatment. The number of melanocytes per field was calculated in these sections. A "pigmentation factor" was defined as the average surface area of silver deposits within an individual melanocyte, multiplied by the number of pigmented melanocytes per field. A value of one was assigned to untreated controls, and values of treatment groups were normalized to their relevant controls. Using the Image Pro system, surface area of silver nitrate deposits and number of melanocytes were measured for whole equivalents. A value of one was assigned to untreated controls and values of treatment groups were normalized to their relevant controls.

Figure 1A is a graph depicting the increase or decrease in relative pigmentation, as measured and calculated by the whole equivalent/Image Pro system, as set forth above, when exposed to benzaldehyde (50□M), hydroquinone (50□M) and UVB irradiation (0.12 J/cm^2).

The human epidermal equivalents were also exposed to mixtures of protease inhibitors, said protease inhibitors are set forth in Table A below. The protease inhibitors were available from Boehringer Mannheim of Indianapolis, IN. Complete® Protease Inhibitor Cocktail tablets available from Boehringer Mannheim were used, containing inhibitors of chymotrypsin, thermolysin, papain, pronase, pancreatic extract and trypsin. Soybean trypsin inhibitor ("STI") was available from Sigma and was dissolved in a 50 mg/ml liposome vehicle or in 1x PBS. All other protease inhibitors used in this *in vitro* example were dissolved in 1xPBS. GDL liposomes were prepared as set forth in Niemic, et al., above, with the exception of the following changes: the non-ionic liposomal formulation contained glycerol dilaurate

(Emulsynt GDL, ISP Van Dyk)/cholesterol (Croda)/polyoxyethylene-10-stearyl ether (Brij76, ICI)/polyoxyethylene-9-lauryl ether, as at ratio of 37.5:12.5:33.3:16.7. Hepes buffer, 0.05M, pH 7.4 (Gibco-BRL of Gaithersburg, MD) was used as the aqueous phase in the preparation of the liposomes. These mixtures of protease inhibitors and different combinations of serine protease inhibitors were tested for their ability to affect melanogenesis. As set forth in Figure 1B, some of the serine protease inhibitors, particularly STI (soybean trypsin inhibitor), were very effective in inhibiting melanogenesis.

TABLE A

Test Formulation	Ingredients
Complete®	Total protease inhibitor mixture - x25
Mix-1	Serine Protease inhibitors - 90 µg/mL Phenylmethyl-sulfonyl fluoride ("PMSF") and 50 µg/mL L-1-Chloro-3-[4-tosylamido]-4-phenyl-2-butanone ("TPCK")
Mix-2	Serine protease inhibitors - 0.1 µg/mL aprotinin, 50 µg/mL Soybean trypsin inhibitor ("STI"), 0.5 µg/mL leupeptin and 0.25 µg/mL (L-1-Chloro-3-[4-tosylamido]-7-amino-2-heptanone-HCl) ("TLCK ")
STI	Soybean trypsin inhibitor - 1 mg/ml

Example 2: A Protease-activated Receptor Is Involved In Pigmentation

Example 1 demonstrates that STI reduces pigmentation. STI inhibits trypsin. Because trypsin is known to activate TR and PAR-2, we tested the possible involvement of TR and PAR-2 in pigmentation. MelanoDerm human epidermal equivalents were treated with the TR and PAR-2 agonists and antagonists set forth in Table B below daily for three days. On the fourth day, the samples were harvested, fixed, and DOPA, H&E or F&M staining was performed. Histological and whole-equivalent examination revealed changes in pigmentation following the treatments. Figure 2 depicts the results of this example. As shown therein, the PAR-2 peptide agonist SLIGRL induced pigmentation in individual melanocytes. Treatment with Compound I, an inhibitor of thrombin and trypsin, resulted in decreased pigmentation.

Figure 3 shows the results of the studies set forth in this example, representing the level of pigmentation in MelanoDerm equivalents treated with TR and PAR-2 reagents. SLIGRL, a PAR-2 agonist, dramatically increased pigmentation, indicating that PAR-2 might be involved in pigmentation. Hirudin, a thrombin-specific inhibitor, and TFLLRNPNDK, a TR selective agonist had no effect on pigmentation. However, SFLLRN, a less specific TR agonists, showed a trend of lightening or reducing pigmentation. This indicates that TR is less likely to be involved in pigmentation.

TABLE B

TR and PAR-2 Reagents	Description
Thrombin	Activates TR
Trypsin	Activates TR and PAR-2
TFLLRNPNDK	TR peptide agonist - activates TR only
SLIGRL	PAR-2 peptide agonist - activates PAR-2 only
SFLLRN	TR peptide agonist - activates TR, TR, cross-reacts with PAR-2
FSLLRN	Scrambled peptide - inactive
Hirudin	Specific inhibitor of thrombin
Compound I	Thrombin and trypsin inhibitor

Example 3: A Dose-response Relation Between Protease-activated Receptors Signaling and Melanogenesis

MelanoDerm equivalents were treated with increasing concentrations of SLIGRL, the PAR-2 peptide agonist, at 0, 10 and 50 μM in the same manner as set forth in Example 2. F&M staining was performed in the fourth day. As shown in Figure 4A, increasing concentrations of SLIGRL, the PAR-2 activator, result in increased pigmentation. Trypsin, a PAR-2 activator, has the same effect. Treatment with increasing concentrations of Compound I, the thrombin and trypsin inhibitor, from 0.1pM to 1μM resulted in decreasing pigmentation (see Figure 4A). Pretreatment of the equivalents with UVB irradiation increased melanogenesis, relative to untreated controls. Compound I was able to reduce this UVB-induced pigmentation as well (Fig. 4B). This example demonstrates a dose-response relation for increasing and decreasing pigmentation with the modulation of PAR-2 signaling. This example also demonstrates that Compound I can inhibit pigmentation and prevent UV-induced pigmentation.

Example 4: PAR-2 is Expressed in Keratinocytes, But Not In Melanocytes

PAR-2 and TR expression have been demonstrated previously in keratinocytes and fibroblasts. This example demonstrates that PAR-2 is expressed in keratinocytes, but not in melanocytes. Furthermore, it demonstrates that TR is expressed in both keratinocytes and melanocytes. In order to demonstrate this, MelanoDerm human epidermal equivalents, human primary melanocyte cultures (neonatal and adult, from Clonetics of San Diego, CA) and Cloudman S91 mouse melanoma cells from ATCC of Rockville, MD were grown in culture and total RNAs were extracted using "RNA Stat-60" reagent available from "Tel-Test B", Incorporated as described in Chomczymski, "Single Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-phenol-chloroform extraction," 162 Anal. Biochem. 156-69 (1987). A sufficient amount of RNase-free DNase available from Promega Corporation under the tradename "RQ1 RNase-free DNase" was then added to the extracted RNA from each sample such that each respective product will yield 200ng of DNased-RNA using the procedure set forth in "RNase-free DNase", protocol published by Promega Corporation (May, 1995). The resulting 200ng of DNased-RNA was reverse transcribed ("RT") via the procedure set forth in "Superscript II Reverse Transcriptase" a protocol published by Gibco-BRL (now Life Technologies, Incorporated) (April 1992), using random hexamers such as the random primers which are commercially available from Life Technologies, Incorporated.

The resulting RT products were then amplified via polymerase chain reaction ("PCR") using about a 0.5

unit (per 100 µl reaction) of a thermostable DNA polymerase which is commercially available from Perkin-Elmer-Cetus Corporation under the tradename "Taq polymerase" and about 0.1 µmol/reaction of TR and PAR-2 specific primers as described in Table C and in Marthinuss et al., 1995 which is hereby incorporated herein by reference or of glyceraldehyde-3-phosphate-dehydrogenase (G3PDH) primers, available from Clontech Laboratories, Inc. of Palo Alto, CA in accordance with the procedures set forth in Marthinuss et al., 1995 or in the protocol accompanying the primers from Clontech Laboratories.

The PCR products were then analyzed using 2% agarose/ethidium bromide gels according to methods well-known in the art in order to compare the level of expression of certain genes in keratinocytes and melanocytes. When necessary for better visualization, the resulting PCR products were precipitated with ethanol according to well-known procedures. When primers for G3PDH were used, only 10% of the PCR reaction products were used. An RNA sample from epidermal equivalents that was not reverse-transcribed was used as a negative control for each PCR amplification. The lack of genomic DNA contaminants was indicated by the lack of a band on the relevant lanes in the gels. A human skin RNA sample which was reverse-transcribed was used as a positive control when commercial positive controls were not available. The migration of the RT-PCR products on the gels was always identical to that of the positive controls, and to that of the reported amplimer sizes.

The relative quality of each respective RT-PCR reaction product was then compared by analyzing the mRNA level of G3PDH, a "housekeeping" gene, in each respective product. As illustrated in FIG. 5 and 6, G3PDH gene expression was found to be similar at all the time points examined, which thereby enabled the comparison of the relative levels of gene expression for the desired genes.

Figure 5A shows that, as expected, TR and PAR-2 are expressed in total skin and in the MelanoDerm equivalents ("MD"). However, S91 melanoma cells ("S91") did not express PAR-2 or TR. To investigate this further, we tested primary newborn ("mel-NB") and adult ("mel-A") melanocytes for TR and PAR-2 expression. As shown in Figure 5B, primary human melanocytes express TR but not PAR-2. Therefore, we suggest that PAR-2 agonists and antagonists can interact with keratinocytes, but not with melanocytes, in the MelanoDerm equivalents, and that TR agonists and antagonists could interact with both keratinocytes and melanocytes. A keratinocyte-melanocyte interaction is, therefore, suggested, during which the keratinocyte-PAR-2 signal is converted into a pigmentation end-point.

Table C illustrates some of the DNA primers used, the amount of $MgCl_2$ required for the PCR reaction, and the length of the PCR cycle.

Table C: DNA Primers Utilized in RT-PCR Assay

Primer (See attached Sequence Listing)	Amt. of $MgCl_2$ (mM)	Cycle (min) @ °C	No. of Cycles	DNA Seq. ID No.
Tyrosinase sense TCAGCCCAGC ATCCTTCTTC	1.25	1 @ 94 2 @ 55 3 @ 72	35	1
Tyrosinase antisense CAGCCATTGT TCAAAAATAC-TGTCC	1.25	1 @ 94 2 @ 55 3 @ 72	35	2
TRP-1 sense 5'CCACTCTAA TAAGCCCAAAC	2.5	1 @ 94 2 @ 55 3 @ 72	35	3
TRP-1 antisense 5'CTCAGCCAT TCATCAAAGAC	2.5	1 @ 94 2 @ 55 3 @ 72	35	4
TRP-2 sense 5'AAAAGACAT ACGAGATTGCC	2.5	1 @ 94 2 @ 55 3 @ 72	35	5
TRP-2 antisense 5'CACAAAAAG ACCAACCAAAG	2.5	1 @ 94 2 @ 55 3 @ 72	35	6
Trypsin sense 5'ATCC/TACT CCTGATCCTTA CC	2.5	1 @ 94 2 @ 45 3 @ 72	35	7
Trypsin antisense 5'TGTCATTGT T/CCAGAGTCT /- CT/GC/GC	2.5	1 @ 94 2 @ 45 3 @ 72	35	8
PAR-2 sense - GGGAAAGGGGT TGGGGTAGAA-CCAGGCTTTTC C (5')	2.5	.5 @ 94 1 @ 55 3 @ 72	30	9
PAR-2 antisense - GGCCAACGGCG	2.5	.5 @ 94 1 @ 55 3 @ 72	30	10

ATGTTTGCCTT - CTTCCTGGGG(3')				
TR-sense - CCTCTGAGTGC CAGAGGTACG- TCTACAG (5')	2.5	.5 @ 94 1 @ 55 3 @ 72	30	11
TR- antisense - CCTAAGTTAAC AGCTTTTTGTA T- ATGCTGTTATT CAGG (3')	2.5	.5 @ 94 1 @ 55 3 @ 72	30	12
Thrombin- sense - AACCTGAAGGA GACGTGGAC (3')	2.5	.5 @ 94 1 @ 55 3 @ 72	35	13
Thrombin- antisense - CAGGAGCCCAG AATATGAGTG (5')	2.5	.5 @ 94 1 @ 55 3 @ 72	35	14

Example 5: Keratinocyte-Melanocyte Contact is Required for Compound I Depigmenting Effect

The results of Example 4 suggest that melanocytes alone might not respond to the depigmenting effect of PAR-2 antagonists. Indeed, the level of pigmentation of human primary melanocytes or choleratoxin-induced S91 cells, which is reduced by hydroquinone and benzaldehyde, was not affected by Compound I.

Since PAR-2 is not expressed in melanocytes, we tested the possible requirement of keratinocyte-melanocyte interactions for the depigmenting effect of Compound I. Primary melanocyte cultures were compared to identical cultures plated under epidermal equivalents (EpiDerm, lacking melanocytes) to create a co-culture with no contact between keratinocytes and melanocytes. These were also compared to MelanoDerm equivalents, where melanocytes are present in the basal layer of the equivalent. Cultures were treated for three days with Compound I, with the PAR-2 agonist SLIGRL, and with the TR agonist TFLLRNPNDK, as set forth in Table D, and DOPA stained on the fourth day. In Table D, keratinocytes are indicated by "K", melanocytes are indicated by "M" and lack of keratinocyte-melanocyte contact is indicated as "no K-M contact". As shown in Table D, no effect on pigmentation was observed in primary melanocytes and in co-cultures treated with these agents. In MelanoDerm equivalents, compound I reduced and SLIGRL induced pigmentation, while TFLLRNPNDK had no effect. These

results demonstrate that keratinocyte-melanocyte contact is required for the PAR-2 effect on pigmentation.

TABLE D

Treatment	Melanocytes (no K)	Co-cultures (no K-M contact)	MelanoDerm (K-M contact)
Compound I	no effect	no effect	lightening
SLIGRL	no effect	no effect	darkening
TFLLRNPNDK	no effect	no effect	no effect

Example 6: Compound I Affects Melanocyte Gene Expression

MelanoDerm equivalents were treated with increasing concentrations of the thrombin and trypsin inhibitor, Compound I, or with increasing concentrations of the PAR-2 agonist SLIGRL. RNAs extracted from untreated and Compound I-treated equivalents were analyzed for gene expression by RT-PCR in the manner set forth above in Example 4. Gene-specific primers were designed as set forth in Table C above, and Clontech primers for human G3PDH were used as in Example 4. Melanogenic genes tested for expression level were tyrosinase, TRP-1, and TRP-2.

A dose-dependent decrease in TRP-1 and a dose-dependent increase in TRP-2 mRNA levels were observed in Compound I-treated samples, as shown in Figure 6A. Tyrosinase expression, however, was not affected. These changes correlated with the dose-dependent whitening effect of this inhibitor. Both patterns of gene expression result in a lightening effect. TRP-2 enzyme processes dopaquinone to 5,6-dihydroxyindole carboxylic acid (DHICA), rather than to 5.6-dihydroxyindole (DHI).

This process results in brown, finely dispersed eumelanin, as opposed to insoluble black eumelanin, and results in a lighter skin tone. TRP-1 stabilizes the melanogenic complex, enabling pigment production. Reduced levels of TRP-1 result in reduced tyrosinase activity and reduced pigmentation. Lack of this protein results in albinism. Increasing concentrations of SLIGRL, however, did not affect melanogenic gene expression (Fig. 6B).

TRP-1 and TRP-2 are melanocyte-specific. Compound I inhibits trypsin and thrombin. Hirudin, a specific thrombin inhibitor, had no effect on pigmentation, as seen above in Example 2. Thus, we decided to test whether trypsin and thrombin are expressed in skin. A probe designed to detect both brain and gastric trypsins, as described in Table C, detected the expression of both mRNAs in a total skin mRNA sample available from Invitrogen of Carlsbad, CA, as well as in MelanoDerm equivalents. The same expression pattern was detected for thrombin. Both trypsin and thrombin were not expressed in normal melanocytes (Figs. 5A, B). These data suggest that if trypsin activates PAR-2, it could be produced by the keratinocytes only. As shown in Figure 6A, treatment with Compound I resulted in increased expression of trypsin. SLIGRL, which did not affect melanogenesis gene expression (Fig. 6B) also increased trypsin expression in the equivalents. We conclude that while trypsin is a possible natural activator of PAR-2 in skin and possibly affects pigmentation, its mRNA levels do not correlate with pigmentation. This suggests that another, yet unidentified serine protease, which is inhibited by compound I, STI and the like, is the natural activator

of PAR-2 in the epidermis. Compounds that induce or inhibit this protease would serve as darkening and lightening agents, respectively.

Example 7: Thrombin and Trypsin Inhibitors and PAR-2 Agonists Affect Pigmentation *In Vivo*

Two guinea pigs were treated twice daily, five days/week for seven weeks with Compound I at 1 and 10 μM in 70:30 ethanol:propylene glycol vehicle on one pigmented nipple. The other nipple of each animal was treated with vehicle only and served as a control. Chromameter measurement after seven weeks of treatment revealed a dose-dependent lightening effect of +9.6 L* and nearly 18 L* units respectively. No visible signs of irritation were observed at that time.

Four groups of three guinea pigs each were treated respectively with Compound I, SFLLRN, FSLLRN and SLIGRL at 10 μM, twice daily five days per week for eight weeks. Chromameter measurement after six weeks demonstrates a lightening effect by Compound I and a darkening effect by SLIGRL, the PAR-2 agonist. The results of this example are set forth in Figure 7.

Example 8: Thrombin and Trypsin Inhibitors and PAR-2 Agonists Affect Pigmentation *In vivo*

A Yucatan microswine was treated with Compound I, SFLLRN, FSLLRN and SLIGRL at 10 μM. Each compound was applied to two sites on the pig twice daily, five days per week for eight weeks. After eight weeks of treatment, chromameter measurements were taken. The application of Compound I resulted in a visible lightening effect. The PAR-2 agonist SLIGRL resulted in

a darkening effect as measured by chromameter. SFLLRN and FSLLRN had no significant effects.

Two Yucatan swine were treated for seven and a half weeks, or for ten weeks, twice daily, five days per week, with increasing concentrations of Compound I. Four concentrations of active compound were used, as follows: 0, 10, 50 and 250μM. Two sites per concentration were placed on opposite sides of the swine dorsum. Chromameter measurements were taken before treatment started and every two weeks thereafter. Pictures were taken periodically and at the end of the experiment. A visible lightening effect was observed during the 4th, 5th and 6th weeks of treatment, for the 250, 50 and 10 μM treatments, respectively. By the eighth week, the whitening effect of the two highest doses was similar. These results are illustrated in Figure 8. The chromameter readings (L*, measuring brightness) during the treatment course of one swine are shown in Fig. 9. A saturation effect is observed, which is a time and concentration dependent. This example demonstrates a visual depigmenting effect by Compound I, in the animal model system most resemble pigmented human skin.

At the end of these experiments, biopsies were taken for histological and electron microscopy (EM) analyses. Histological samples were stained with H&E and F&M. H&E staining showed that there was no irritation, inflammatory response or changes in skin architecture, demonstrating the safety of using Compound I over long periods of time. F&M staining demonstrated that there was reduced pigmentation in the treated samples, both in the basal layer and throughout the

epidermis. These results are illustrated in Figure 10. Untreated and vehicle-treated samples (Fig. 10A) were identical and darkest. The 10 μM treatment (Fig. 10B) showed reduced pigmentation and the 50 and 250 μM treatments (Fig. 10C, 10D, respectively) were the lightest.

The results of this example suggest that the maximal whitening effect of Compound I could be achieved with higher concentration over a shorter period of time or with lower concentration over a longer period of time. Thus, at least two difference regimens may be used to achieve the desired skin whitening results.

Example 9: Ultrastructural Studies Demonstrate the Effect of Compound I On the Skin *In Vitro* and *In Vivo*

Ultrastructural analysis was performed on MelanoDerm equivalents and swine skin sites treated with Compound I. MelanoDerm equivalents treated with Compound I were analyzed for melanosome formation and distribution using electron microscopy. Treated samples contained more melanosomes, but less mature melanosomes, i.e., melanosomes which evidence reduced melanin production, within the melanocytes, relative to untreated controls (Fig. 11A, 11B). Dendrites containing melanosomes were easily identified within treated keratinocytes (Fig. 11C), but were difficult to find within control keratinocytes. This suggests abnormal melanosome formation and slow or impaired melanosome transfer into keratinocytes in the treated samples.

Skin samples from Yucatan swine treated with compound I for eight weeks, as described in example 8,

were also analyzed by electron microscopy. Melanosomes within keratinocytes of treated sites were smaller and less pigmented, compared to controls (Fig.11D, 11E and 11F). Moreover, the distribution of melanosomes within the treated skins was abnormal. Melanosomes were detected mainly at the epidermal-dermal border, compared to a random distribution in untreated controls (Fig. 11G, 11H). While we cannot rule out other mechanisms, we suggest that Compound I treated keratinocytes were unable to actively take or receive melanosomes from the presenting dendrites.

Example 10: The *in vivo* depigmenting effect of Compound I is reversible

A Yucatan swine was treated with Compound I, 250 □M, for eight weeks, twice daily, five days a week, on eight sites. All sites showed visible depigmentation by the end of the treatment period, as set forth in Figure 12B. For the following four weeks (starting at week nine of the experiment), the color of the treated sites was monitored, and two biopsies were taken each week from two treated sites. Untreated sites were biopsied as well. The depigmenting effect could be visualized at one and two weeks post treatment, and a complete reversal was observed by the forth week. Histological examination of F&M stained skin sections confirmed the repigmentation observed visually (as indicated in Fig. 12). As early as one week post treatment, repigmentation was demonstrated histologically. The visual observations correlate with the histological demonstration of stratum corneum pigmentation. This example demonstrates that Compound I does not induce a permanent damage to the pigmentation

machinery, and its effect is reversible *in vivo*. Figure 12A shows two histological F&M stained sections of sites which were not treated with Compound I. Figure 12B shows two histological F&M stained sections of sites which were treated with Compound I for eight weeks. Figure 12C shows sections of sites which were treated for eight weeks with Compound I, one week after treatment was stopped. Figure 12D shows sections of sites which were treated for eight weeks with Compound I, two weeks after treatment was stopped. Figure 12E shows sections of sites which were treated for eight weeks with Compound I, four weeks after treatment was stopped. As indicated in Figure 12E, the sections were fully repigmented four weeks after the end of treatment.

Example 11: Preparation of naturally-derived products containing STI

Example 1 demonstrates that the presence of soybean trypsin inhibitor in any lightening formulation is desirable for its depigmenting activity. Based on analytical testing, it has been determined that soybean milk and soybean paste are rich sources of soybean trypsin inhibitor.

To make soybean paste, soybeans were first soaked in deionized or purified water for several hours. The soybeans were ground after they were fully hydrated, with the addition of small quantities of water, if needed, to smoothen the paste. To make soybean milk, the same procedure was performed with the addition of more water. (The grinding process allows the soybean milk to be extracted). After collection, the soybean milk was filtered to remove any residual parts of the bean husk.

Soybean milk, soybean paste and miso were prepared to be used as naturally-derived materials that contain STI and are able to lighten skin color.

Example 12: Treatment With Naturally-Derived Materials that affect the PAR-2 Pathway Induces Depigmentation

Two Yucatan swine were treated for eight and ten weeks, twice a day, five days a week, with different soybean- and lima-bean-derived products. These natural products include soybean paste, soybean protein acid hydrolysate, miso, native and boiled soybean milk, and a commercially available extract of soybean (Actiphyte™ of Active Organics, Dallas Texas), as well as purified STI, and different preparations of trypsin inhibitors from soybeans and limabeans. At seven weeks of treatment, all sites were visually lighter than the surrounding skin, except for the boiled soybean milk and the soybean protein acid hydrolysate treated sites. Histological analysis of biopsies from the treated sites following F&M staining confirmed the depigmenting effect of the soybean and limabean products. An example of such histological data is given in Fig. 13. The lack of depigmenting activity in the boiled soybean milk and in the soy protein acid hydrolysate is explained by the denaturation or the degradation of the soy proteins in these preparations, respectively. We theorize that the active depigmenting agents in the soybean and limabean products are soybean trypsin inhibitor (STI) and limabean trypsin inhibitor, respectively. (Example 1 shows the depigmenting effect of STI *in vitro*). This example demonstrate that natural extracts containing trypsin inhibitory activity could be used as whitening agents which affect the PAR-2 pathway.

Example 13: An STI in liposome formulation can lighten human age spots

An individual with three age spots on the dorsum of their hand was treated for eight weeks, twice a day, with the following: The age spot located closest to the arm was treated with placebo, containing 20 mg/ml of liposomes. The middle age spot was not treated. The third age spot was treated with STI, 1%, in liposomes (20 mg/ml).

GDL liposomes were prepared as set forth in Niemiec, et al., above, with the exception of the following changes: the non-ionic liposomal formulation contained glycerol dilaurate (Emulsynt GDL, ISP Van Dyk)/cholesterol (Croda)/polyoxyethylene-10-stearyl ether (Brij76, ICI)/polyoxyethylene-9-lauryl ether, as at ratio of 37.5:12.5:33.3:16.7. Hepes buffer, 0.05M, pH 7.4 (Gibco-BRL of Gaithersburg, MD) was used as the aqueous phase in the preparation of the liposomes. UV and visible light digital pictures were taken at time 0, 4 and 8 weeks of treatment. L* (brightness) values were calculated from the images using Adobe Photoshop.

As shown in figure 14, the age spot treated with STI became lighter following 8 weeks of treatment. Figure 14 is a composite of four pictures. The left panel is the visible light pictures of the hand, before (upper) and after (lower) 8 weeks of treatment. At this orientation the top age spot is the placebo-treated, the middle age spot is untreated, and the lower age spot is the STI-treated. The right panel shows the same hand at the same time points, using UV-photography. UV light enables the visualization of pigment deeper in the skin, demonstrating that the STI whitening effect was not superficial. Figure 14

clearly demonstrates that the STI formulation was able to lighten the lower age-spot. An increase of 15 L* units was calculated for this STI-treated site, further demonstrating the ability of this treatment to lighten age spots.

Example 14: Depigmenting formulations with soybean milk

In making the soybean milk, it was discovered that the rich emolliency of the milk would be desirable in a skin care formulation. Because water is used as the predominant ingredient of any oil-in-water emulsion, and in many other skin-care formulations we hypothesized that the soymilk could be used to substitute for the deionized water in such formulations. However, we expected that this type of formulation would not be physically stable due to the immiscibility of the oil and water components of the soybean milk. Surprisingly, we found that this substitution of soybean milk for water was physically stable. Formulations utilizing soybean milk should contain between about 1% and about 99% of soybean milk, more preferably from about 80% to about 95% soybean milk. Preferably, this and similar formulations should include a viscosity builder in an amount from about 0% to about 5% (more preferably, from about 0.1 to about 2%), one or more emollients in an amount up to about 20% and/or emulsifiers in an amount from about 0.1% to about 10% (more preferably from about 3 to about 5%), and, optionally, a spreading agent in an amount from about 0 to about 5% (more preferably from about 1 to about 2%), a preservative, a chelating agent or a humectant. The preservative should be present in an

effective amount in order to preserve integrity of the milk and maintain the composition's activity. Sufficient thickener should be present to impart body to the formulation without causing it to become so viscous that it would hinder spreadability, e.g., from about 0 to about 10%, more preferably from about 3 to about 5%. Sunscreen, antioxidants, vitamins other depigmenting agents and other skin care topical ingredients may also be incorporated into the compositions of this invention.

A particularly preferred example of a depigmenting formulation substituting soymilk for water is shown in table E below.

TABLE E

Ingredient	Function	% Wgt/Wgt
soybean milk	Vehicle, depigmenting	84.9%
aluminum starch octenyl succinate	viscosity builder	0.75%
cyclomethicone	spreading agent	2%
PEG 6-capric/caprylic triglycerides	emollient/emulsifier	3%
phenoxyethanol	preservative	0.75%
sucrose cocoate	emollient/emulsifier	1%
Na_2EDTA	chelating agent	0.1%
glycerin	humectant	2.5%
polyacrylamide; isoparaffin; laureth-7	thickener	5%

STI, soybean paste and other trypsin inhibitor-containing natural extracts can be incorporated into such formulations to provide increasing concentrations of the serine protease inhibitor. Use levels of the added active ingredient can range between 0.01% to 15% in a formulation. Other depigmenting agents, including PAR-2 inhibitors, tyrosinase inhibitors, hydroquinones, soy products, ascorbic acid and its derivatives, as

well as other ingredients with skin care benefits could also be incorporated into this formulation.

Example 15: An Oil-in-water Emulsion depigmenting formulation

Two examples of a depigmenting formulation with oil-in-water emulsion are presented in Table F. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 4 of Table F. A similar formulation with Compound I is presented in column 5 of Table F. Compound I in this composition could be replaced with similar compounds, or with serine protease inhibitors or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also listed in Table F. The deionized water content of these formulations could be replaced with soybean milk.

Table F

CTFA Name	Function	%W/W	%W/W	Ranges
Cetearyl Glucoside	Surfactant	1.4	1.4	0.1-2.8
C12-15 Alkyl Benzoate	Surfactant	4.0	4.0	1-6
Octyl Hydroxystearate	Emollient	1.0	1.0	0-5
Dimethicone	Spreading Agent	1.0	1.0	0-5
Cyclomethicone	Spreading Agent	1.0	1.0	0-5
Cetyl Alcohol	Emollient	2.5	2.5	0-4
Butylated Hydroxytoluene	Anti-oxidant	0.1	0.1	0-0.5
Octyl Methoxycinnamate	Sunscreen	6.0	6.0	0-10
Propylparaben	Preservative	0.5	0.1	0-0.5
Vitamin E acetate	Anti-oxidant	0.5	0.5	0-0.5
Tocopherol Acetate	Anti-oxidant	0.5	0.5	0-0.5
Glycerine	Humectant	3.0	3.0	0-20
D-Pathenol	Pro-Vitamin	0.5	0.5	0-5
Disodium EDTA	Chelator, whitening agent	0.1	0.1	0.01-1
Methyl Paraben	Preservative	0.2	0.2	0-0.3
Carbomer	Thickener	0.35	0.35	0-3
Deionized Water or Soybean Milk	Carrier / Whitening Agent	76.35	77.5	50-80
STI or natural extract	Whitening Agent	1.0	0	0-15
Compound I	Whitening Agent	0	0.25	0-1

To prepare this formulation, the ingredients of the lipid phase were combined and mixed at 85°C, and then cooled to 60°C. In a separate vessel, the carbopol was slowly added to the water or to the soybean milk. After mixing for ten minutes the rest of the aqueous phase ingredients were added and the mix was heated to 60°C. The two phases were then combined, mixed for ten minutes, and cooled to room temperature. Of course, one or more depigmentation agents may be combined within the same formulation, in this Example and in the following examples and other embodiments of the methods and compositions of this invention.

Example 16: Depigmenting Composition (Oil-in-Water Emulsion)

Two additional examples of an oil-in-water emulsion depigmenting formulation are presented in Table G. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 3 of Table G. A similar formulation with Compound I is presented in column 4 of Table G. Compound I in this composition could be replaced with similar compounds or with serine protease inhibitor or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also listed in Table G. The deionized water content of these formulations could be replaced with soybean milk.

Table G

CTFA Name	Function	%W/W	Pref'd. Ranges
Ethanol	Solvent	12.0	5-20
Propylene Glycol	Solvent	3.0	1-10
Hydroxyethylcellulose	Thickener / Polymer	0.2	0-3
Acrylates/ C10-30 Alkyl Acrylate Crosspolymer	Thickener / Polymer	1.0	0-3
Panthenol (98%)	Pro-Vitamin / Humectant	1.5	0.1-3
Fragrance	Fragrance	0.5	0-0.5
Isohexadecane	Spreading Agent	4.0	0-5
Vitamin E acetate	Anti-oxidant	1.0	0-2
Sodium Hydroxide	Neutralizer	0.35	0.1-0.5
Glycerine	Humectant	3.0	0-20
Deionized Water or Soybean Milk	Carrier / Whitening Agent	71.95	60-80
Compound I	Whitening Agent	0.25	0-1
STI or natural extract	Whitening / Agent	0	0-15

To prepare this formulation, the hydroxyethylcellulose was slowly added to the water or to the soybean milk and stir until completely dissolved. In a separate container the Acrylates/ C10-30 Alkyl Acrylate Crosspolymer was added and stir until completely dissolved. The content of the two containers was combined and mixed for 20 minutes. Vitamin E acetate was then added and mixed, following by the addition of Isohexadecane and Panthenol (98%). After mixing for five minutes the STI, or the natural extract, or Compound I were added together with Propylene Glycol, and stirred for 5 minutes. Next, glycerine was added and the formulation was stirred for 20 minutes. Finally, the pH was adjusted with sodium hydroxide to 8 for STI (range is 6-8.5) or to 7 for Compound I (range is 5.5-8.5).

Example 17: Depigmenting Composition (Water-In-Oil Emulsion)

An example of a depigmenting formulation with water-in-oil emulsion is presented in Table H. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 4 of Table H. A similar formulation with Compound I is presented in column 5 of Table H. Compound I in this composition could be replaced with similar compounds or with serine protease inhibitor or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also

To prepare this formulation, the hydroxyethylcellulose was slowly added to the water or to the soybean milk and stir until completely dissolved. In a separate container the Acrylates/ C10-30 Alkyl Acrylate Crosspolymer was added and stir until completely dissolved. The content of the two containers was combined and mixed for 20 minutes. Vitamin E acetate was then added and mixed, following by the addition of Isohexadecane and Panthenol (98%). After mixing for five minutes the STI, or the natural extract, or Compound I were added together with Propylene Glycol, and stirred for 5 minutes. Next, glycerine was added and the formulation was stirred for 20 minutes. Finally, the pH was adjusted with sodium hydroxide to 8 for STI (range is 6-8.5) or to 7 for Compound I (range is 5.5-8.5).

Example 17: Depigmenting Composition (Water-In-Oil Emulsion)

An example of a depigmenting formulation with water-in-oil emulsion is presented in Table H. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 4 of Table H. A similar formulation with Compound I is presented in column 5 of Table H. Compound I in this composition could be replaced with similar compounds or with serine protease inhibitor or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also

listed in Table H. The deionized water content of these formulations could be replaced with soybean milk.

Table H

Phase	CTFA Name	Function	%W/W	%W/W	Pref'd Ranges
OIL	Mineral Oil	Emollient	25.0	25.0	40-80
	Sorbitan Monooleate	Surfactant	5.0	5.0	1-6
	Stearyl Alcohol	Emollient	25.0	25.0	20-60
	Dimethicone	Spreading Agent	1.0	1.0	1-5
	Cetyl Alcohol	Emollient	2.0	2.0	0.1-10
	Hydrogenated Lecithin	Anti-oxidant	3.0	3.0	0-10
	Parsol MCX	Sunscreen	3.0	3.0	0-10
	Propylparaben	Preservative	0.5	0.5	0.01-0.5
	Vitamin E acetate	Anti-oxidant	0.5	0.5	0.01-0.5
AQUEOUS	Glycerine	Humectant	3.0	3.0	0-20
	Methyl Paraben	Preservative	0.2	0.2	0.01-0.3
	Water or Soy Milk	Carrier / Whitening Agent	30.8	31.55	20-45
	STI	Whitening Agent	1.0	0	0-10
	Cpd I	Whitening Agent	0	0.25	0-1

To prepare this formulation the stearyl alcohol and mineral oil were melted at 70°C. The other oil phase ingredients were added and the mixture heated to 75°C. The aqueous phase ingredients, which have been previously dissolved in the bulk phase water or Soy Milk and warmed to 70°C, were then added and the mixture was stirred until it congealed.

Example 18: Depigmentation Composition (Aqueous Gel)

Two examples of a depigmenting formulation with aqueous gel are presented in Table J. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 3 of Table J. A similar formulation with

Compound I is presented in column 4 of Table J. Compound I in this composition could be replaced with similar compounds or with serine protease inhibitor or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also listed in Table J. The deionized water content of these formulations could be replaced with soybean milk.

TABLE J

CTFA Name	Function	%W/W	%W/W	
Octoxynol-13	Surfactant	0.2	0.2	0.05-0.5
2,4-Hexadienoic Acid	Preservative	0.1	0.1	0-0.3
Benzenemethanol	Preservative	1.0	1.0	0-2
Disodium EDTA	Chelator / Preservative	0.05	0.05	0.01-0.2
Ascorbic Acid	Anti-oxidant	0.1	0.1	0-0.2
Sodium Metabisulfite	Anti-oxidant	0.2	0.2	0-0.3
Carbomer	Thickener	1.5	1.5	0-3.0
NaOH %20 Soln.	Neutralizer	2.45	2.45	0.1-5
DEIONIZED Water or Soybean Milk	Carrier / Whitening Agent	93.4	94.15	85-98
STI or natural extract	Whitening Agent	1.0	0	0-15
Compound I	Whitening Agent	0	0.25	0-1

listed in Table K. The deionized water content of these formulations could be replaced with soybean milk

Table K

CTFA Name	Function	%W/W	Range
Ethanol	Solvent (1)	70	40-90
Propylene Glycol	Solvent (2)	29	1-40
Deionized Water	Carrier	q.s.	1-40
STI	Whitening Agent	0	
Compound I	Whitening Agent	1 μM	.00001 - 1

To prepare this formulation Compound I was dissolved in water. The ethanol and propylene glycol were mixed and combined with the aqueous solution containing Compound

I. In summary, we have demonstrated that activation of the keratinocyte receptor PAR-2 results in increased pigmentation. Preferably, such activation may be accomplished by the use of trypsin or SLIGRL or SLIGKVD or other SLIGRL or SLIGKVD derivatives. We have also demonstrated that whitening may be accomplished by the use of serine protease inhibitors or PAR-2 antagonists, as well as by melanosome-transfer blockers. Other compounds known to those of skill in the art that inhibit melanosome transfer into keratinocytes could also be used as depigmenting agents.

Compound I, a trypsin and thrombin inhibitor, for example, inhibits melanosome transfer to keratinocytes. STI works by the same mechanism. The accumulation of undelivered melanosomes in the melanocytes could induce a negative feed back mechanism, that slows new melanosome formation. The production of TRP-1, the major glycoprotein in melanocytes, is down-regulated, which leads to destabilization of tyrosinase. This results in reduced melanin formation, and in a color

switch to a lighter brown, as the ratio of TRP-1:TRP-2 is reduced. The melanosomes accumulation in the melanocyte after Compound I treatment, or after STI treatment, therefore, have reduced and altered melanin content, which adds to the whitening effect of compound I or STI.

WHAT IS CLAIMED IS:

1. A method of effecting changes in mammalian skin pigmentation comprising administering to a mammal a pigmentation-changing effective amount of a compound which affects the PAR-2 pathway.

2. A method according to claim 1 wherein said compound inhibits the PAR-2 pathway.

3. A method according to claim 2 wherein said compound is an antagonist of PAR-2.

4. A method according to claim 3 wherein said compound binds to or blocks but does not activate PAR-2.

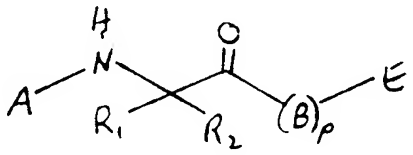
5. A method according to claim 4 wherein said compound is selected from the group consisting of antagonists based on SLIGRL which bind to or block but do not activate PAR-2, antagonists based on SLIGKVD which bind to or block but do not activate PAR-2 and mixtures thereof.

6. A method according to claim 2 wherein said compound is a protease inhibitor.

7. A method according to claim 6 wherein said compound is a serine protease inhibitor.

8. A method according to claim 7 wherein said compound is a thrombin and/or tryptase and/or trypsin inhibitor.

9. A method according to claim 8 wherein said compound is a compound of formula I:



wherein:

A is selected from the group consisting of C_{1-8}alkyl, carboxyC_{1-4}alkyl,

C_{1-4}alkoxycarbonlyC_{1-4}alkyl, phenylC_{1-4}alkyl, substituted phenylC_{1-4}alkyl (where the phenyl substituents are independently selected from one or more of, C_{1-4} alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4} alkoxycarbonyl), formyl, C_{1-4}alkoxycarbonyl, C_{1-2}alkylcarbonyl, phenylC_{1-4}alkoxycarbonyl, C3-7cycloakylcarbonyl, phenylcarbonyl, substituted phenylcarbonyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4} alkoxycarbonyl), C_{1-4}alkylsulfonyl, C_{1-4}alkoxysulfonyl, perfluoroC_{1-4}alkyl-sulfonyl, phenylsulfonyl, substituted phenylsulfonyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4} alkoxycarbonyl), 10-camphorsulfonyl, phenylC_{1-4}alkysulfonyl, substituted phenylC_{1-4}alkysulfonyl, C_{1-4}alkylsulfinyl, perfluroC_{1-4}alkylsulfinyl, phenylsulfinyl, substituted phenylsulfinyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), phenylC_{1-4}alkylsulfinyl, substituted pehnylC_{1-4}alkylsulfinyl, 1-naphthylsulfonyl, 2-naphthylsulfonyl or substituted naphthylsulfonyl (where the naphthyl substituents are independently selected from one or more of, C_{1-4}alkyl,perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, carboxy or C_{1-4}alkoxyy-carbonyl), 1-naphthylsulfinyl, 2-naphthylsulfinyl or substituted naphthylsulfinyl (where

the naphthyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl);

a D or L amino acid which is coupled as its carboxy terminus to the nitrogen depicted in formula I and is selected from the group consisting of alanine, asparagine, 2-azetidinecarboxylic acid, glycine, N-C_{1-8}alkyglycine, proline, 1-amino-1-cycloC_{3-8}alkylcarboxylic acid, thiazzolidine-4-carboxylic acid, 5,5-dimethylthiazolidine-4-carboxylic acid, oxadolidine-4-carboxylic acid, pipecolinic acid, valine, methionine, cysteine, serine, threonine, norleucine, leucine, tert-leucine, isoleucine, phenylalanine, 1-naphthalanine, 2-naphthalamine, 2-thienylalanine, 3-thienylalanine, [1,2,3,4]-tetrahydroisoquinoline- 1-carboxylic acid and 1,2,3,4,]-tetrahydroisoquinoline-2-caroboxylic acid where the amino terminus of said amino acid is connected to a member selected form the group consisting of C_{1-4}alkyl,tetrazol-5yl-C_{1-2}alkyl, carboxytC_{1-4}alkyl, C_{1-4}alkoxycarbonylC14alkyl, phenylC_{1-4}alkyl, substituted phenyl C_{1-4} alkyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboy or C_{1-4}alkoxycarbonyl), 1,1-diphenylC_{1-4}alkyl, 3-phenyl-2-hydroxypropionyl, 2,2-diphenyl- 1-hydroxyethylcarbonyl, [1,2,3,4]-tetrahydroisoquinoline- 1-carbonyl, [1,2,3,4]-tetrahydroisoquinoline-3,carbonyl, 1-methylamino-1-cyclohexanecarbonyl, 1-hydroxy-1-cyclohexanecarbonyl, 1-hydroxy-1-pheny-lacetyl, 1-cyclohexyl-1-

hydroxyacetyl, 3-phenyl-2-hydroxypropionyl, 3,3-diphenyl-2-hydroxypropionyl, 3-cyclohexyl-2-hydroxypropionyl, formyl, C_{1-4}alkoxycarbonyl, C_{1-12}alkylcarbonyl, perfluoroC_{1-4}alkyl, C_{1-4}alkylcarbonyl, phenylC_{1-4}alkylcarbon1, substituted phenylC_{1-4}alkylcarbonyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo amido, nitro amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl) 1,1-diphenylC_{1-4}alkylcarbonyl, substituted 1,1-diphenylC_{1-4}alkylcarbonyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoro C_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4} alkoxy-carbonyl), perfluoroC_{1-4}alkysulfonyl, C_{1-4}alkysulfonyl, C_{1-4}alkoxysulfonyl, phenysulfonyl, substituted phenylsulfonyl (where the phenyl substituents are independently selected from one or more of, C-1alkyl, perfluoro C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), 10-cxamphorsulfonyl, phenylC_{1-4}alkylsulfonyl, substituted phenylC_{1-4}alkylsufonyl, perfluroC_{1-4}alkysulfinyl, C-14alkysulfinyl, phenylsulfinyl, substituted phenysulfinyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoro C_{1-4}alkyl, C_{1-4} alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4} alkylamino, C_{1-4} dialkylamino, carboxy or C_{1-4} alkoxycarbonyl), 1-naphthysulfon1, 2-naphthylsulfonyl, substituted naphthylsulfonyl (where the naphthyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboy or C_{1-}

$_4$alkoxycarbonyl),1-naphthysulfinyl, 2-naphthysulfinyl, and substituted naphthylsulfinyl (where the naphthyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo amido, nitro, amino, C_{1-4}alkylamino, C104dialkylamono, carboxy or C-14alkoxycarbonyl):
or a poly peptide comprised of two amino acids, where the first amino acid is a D or L amino acid, bound via its carboxy terminus tot he nitrogen depicted in Formula I and is selected from the group consisting of glycine, N-C_{1-8}alkylglycine, alanine, 2-azetidinecarboxylic acid, proline, thiazolidine-4-carboxylic acid, 5.5-dimethylthiazolidine-4-carboxylic acid, oxazolidine-4-carboxylic acid, 1-amino-1-cycloC_{3-8} alkylcarboxylic acid, 3-hydroxypropoline, 4-hydroxyproline, 3-(C_{1-4}alkoxy)proline, 4(C_{1-4}alkoxy)proline, 3,4-dehydroprline, 2,2-dimethyl-4-thiazolidine carboxylic acid, 2.2-dimethyl-4-oxadolidine carboxylic acid, pipecolinic acid, valine, methionine, cysteine, asparagine, serine, threonine, leucine, tert-leucine, isoleucine, phenylalanine, 1-naphthalanine, 2-naphthalanine, 2-thienylalanine, 3-thienylalnine, [1,2,3,4]-tetrahydroisoquinoline- 2-carboxylic acid, aspartic acid-4-C_{1-4}alkyl ester and glutamic acid 5-C_{1-4}alkyl ester
and the second D or L amino acid, is bound to the amino terminus of said first amino acid, and is selected from the group consisting of phenylalanine, 4-benzolyphenylalanine, 4-carboxyphenylalanine, 4-(Carboxy C1-2alkyl)phenylalanine, substituted phenylalanine (where the phenyl substituents are independently selected from one or more of C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy

or C_{1-4}alkoxycarbonyl), 3-benzothienylalanine, 4-biphenylalanine, homophenylalanine, octahydroindole-2-carboxylic acid, 2-pyridylalanine, 3-pyridylalanine, 4-thiazolyalanine, 2-thienylalanie, 3-(3-benzothienyl)alanine, 3-thienylalanine, tryptophan, tyrosine, asparagine, 3-tri-C_{1-4}alkylsilylalanine, cyclohexylglycine, diphenylglycine, phenylglycine, methionine sulfoxide, methionine sulfone, 2,2-dicyclohexylalanine, 2-(1-naphthylalanine), 2-(2-naphthylalanine), phenyl substituted phenylalanine (where the substituents are selected from C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4} alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialylamino, carboxy or C_{1-4} alkoxycarbonyl), aspartic acid, aspartic acid-4C_{1-4}alkyl, perfluoroc_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbony), aspartic acid, aspartic acid-4-C_{1-4}alkyl ester glutamic acid, glutamic acid-5-C_{1-4} alkyl ester, cycloC3-salkylaalanine, substituted cycloC_{3-8}alkylalanine (where the ring substituents are carboxy, C_{1-4} alkyl ester, cycloC3-salkylalanine, substituted cycloC_{3-8}alkylalanine (where the ring substituents are carboxy, C_{1-4}alkylcarboxy, C_{1-4}alkoxycarbonyl or aminocarbonyl), 2,2-diphenylalanine and all alpha-C_{1-5}alkyl of all amino acid derivatives thereof, where the amino terminus of said second amino acid is unsubstituted or monosubstituted with a member of the group consisting of formyl, C1-12 alkyl, tetrazol-5-ylC1-2alkyl, carboxyC1-8 alkyl, carboalkoxyC_{1-4}alkyl, phenyl C_{1-4}alkyl, substituted phenylC_{1-4}alkyl (where the phenyl substituents or independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-}

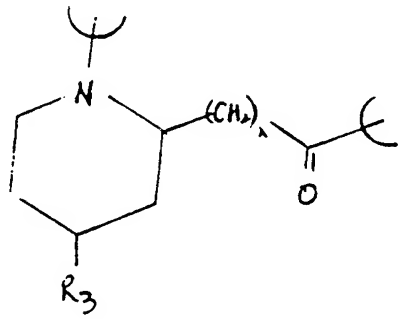
$_4$dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), 1,1-dipehnylC_{1-4}alkyl, C1-6alkoxycarbonyl, phenylC1-6alkoxycarbonyl, C1-2alkylcarbonyl, perfluoroC_{1-4}alkylCo-4alkylcarbonyl, phenyC_{1-4}alkylcarbonyl, substituted phenyC_{1-4}alkylcarbonyl(where the phenyl substituents are independently selected from one or more of C_{1-4}alkyl, perfluoro C_{1-4}alkyl, C_{1-4} alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), 1,1-diphenylC_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxycarbonyl), 10-camphorsulfonyl, phenylC_{1-4}alkysulfonyl, substituted phenylC_{1-4}alkylsulfonyl, C_{1-4}alkysulfinyl, perfluoro C_{1-4}alkylsulfinyl, phenylsulfinyl, substituted phenylsulfinyl (where the phenyl substituents are independently selected from one or more of, C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamono, C_{1-4}dialkylamono, carboxy or C_{1-4}alkoxycarbonyl), phenyC_{1-4}alkylsulfinyl, substituted phenylC_{1-4}alkylsulfinyl 1-naphthylsulfonyl, 2-naphthylsulfonyl, substituted naphthylsulfonyl (where the naphthyl substituent is selected from C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy or C_{1-4}alkoxycarbonyl), 1-haphthyl-sulfinyl, 2-haphthylsulfinyl and substituted naphthyl-sulfinyl (where the naphthyl substituent is selected from C_{1-4}alkyl, perfluoroC_{1-4}alkyl, C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C-14dialkylamino, carboxy or C_{1-4}alkoxycarbonyl); R_1 is selected from the group consisting of hydrogen and alkyl;

R_2 is selected from the group consisting of aminoC2-salkyl, guanidinoC_{2-5}alkyl, C_{1-4}alkylguanidinoC_{2-5}alkyl,diC_{1-4}alkylguanidinoC_{2-5}alkyl, amidinoC_{2-5}alkyl, C_{1-}

$_4$alky-lamidinoC_{2-5}alkyl, diC_{1-4}alky-lamidinoC_{2-5}alkyl, C_{1-3}alkoxyC_{2-5}alkyl, phenyl, substituted phenyl (where the substituents are independently selected from one or more of amino, amidino, guanidino, C_{1-4}alkylamino, C_{1-4}dialkylamino, halogen, perfluoro C_{1-4}alkyl, C_{1-4}alkyl, C_{1-3} alkoxy or nitro), benzyl, phenyl substituted benzyl (where the substituents are independently selected from one or more of, amino, amidino, guanidino, C_{1-4}alkylamino, C_{1-4}dialky-lamino, halogen, perfluoro C_{1-4}alkyl, C1-04alkyl, C_{1-3}alkoxy or nitro), hydroxyC_{2-5}alkl, C`$_{1-5}$alkylaminoC_{2-5}alkyl, C_{1-5}dialkylaminoC_{2-5}alkyl, 4-aminocyclohexylC_{0-2}alkyl and C_{1-5}alkyl;

p is 0 or 1;

B is



where n is 0-3, R_3 is H or C1-5alkyl and the carbonyl moiety of B is bound to E;

E is a heterocycle selected from the group consisting of oxazolin-2-yl, oxazol-2-yl, thiazol-2-yl, thiazol-5-yl, thiazol-4-yl, thiazolin-2-yl, imidazol-2-yl, 4-oxo-2-quinoxalin-2yl, 2-pyridyl, 3-pyridyl, benzo[b}thiophen-2-yl, triazol-4-yl triazol-6-yl, pyrazol-2-yl, 4,5,6,7-tetrahydrobenzothiazol-2yl, naphtho[2,1-d]thiazol-2-yl, naphtho[1-2-d]thiazol-2-yl quinoxalin- 2-yl, isoquinolin-1-yl, isoquinolin-3-yl, benzo [b]furan-2-yl, [pyrazin-2-yl, quinazolin-2-yl, isothiazol-5-yl, isothiazol-3-yl, purin-8yul and a substituted heterocycle where the substituents are

selected from C_{1-4} from C-14alky, perfluoro C_{1-4}alkyl,C_{1-4}alkoxy, hydroxy, halo, amido, nitro, amino, C_{1-4}alkylamino, C_{1-4}dialkylamino, carboxy, C_{1-4}alkoxycarbonyl, hydroxy or phenylC_{1-4} alkylaminocarbonyl;

or pharmaceutically acceptable salts thereof.

10. A method according to claim 9 wherein said compound contains a d-phenylalanine-proline-arginine sequence.

11. A method according to claim 10 wherein said compound is (S)-N-Methyl-D-phenylalanyl-N-[4-[(aminoiminomethyl)amino]-1-(2-benzothiazolylcarbonyl)butyl]-L-prolinamide.

12. A method according to claim 6 wherein said compound is a natural product which affects the PAR-2 pathway.

13. A method according to claim 12 wherein said compound is derived from one or more of the botanical families leguminosae, solanaceae, gramineae and cucurbitaceae.

14. A method according to claim 13 wherein said compound is derived from legumes.

15. A method according to claim 14 wherein said compound is selected from the group consisting of: undenatured soybean extract, limabean extract, blackbean extract or mixtures thereof.

16. A method according to claim 15 wherein said compound is selected from the group consisting of: fractions of undenatured soybean extract, limabean extract, blackbean extract and mixtures thereof.

17. A method according to claim 14 wherein said compound is selected from the group consisting of soybean milk, limabean milk, blackbean milk, soybean extract, limabean extract, blackbean extract, soybean

paste, limabean paste and blackbean paste and mixtures thereof.

18. A method according to claim 2 wherein said compound is a melanosome transfer inhibitor.

19. A method according to claim 1 wherein said compound activates the PAR-2 pathway.

20. A method according to claim 19 wherein said compound is a PAR-2 agonist which binds to and activates PAR-2.

21. A method according to claim 20 wherein said compound is selected from the group consisting of SLIGRL, SLIGKVD and derivatives of SLIGRL and SLIGKVD which bind to and activate PAR-2 and mixtures thereof.

22. A method according to claim 19 wherein said compound is a protease which activates PAR-2.

23. A method according to claim 22 wherein said compound is a serine protease which activates PAR-2.

24. A method according to claim 23 wherein said compound is selected from the group consisting of trypsin, tryptase, thrombin and proteases naturally-occurring in the skin which activates PAR-2.

25. A method according to claim 1 wherein said compound is a melanosome transfer enhancer.

26. A composition for affecting changes in mammalian skin pigmentation comprising a pigmentation-changing effective amount of a compound which affects the PAR-2 pathway.

27. A composition according to claim 26 wherein said compound is a PAR-2 pathway inhibitor.

28. A composition according to claim 27 wherein said compound is an antagonist of PAR-2.

29. A composition according to claim 28 wherein said compound binds to or blocks but does not activate PAR-2.

30. A composition according to claim 29 wherein said compound is selected from the group consisting of antagonists based on SLIGRL which bind to or block but do not activate PAR-2, antagonists based on SLIGKVD which bind to or block but do not activate PAR-2 and mixtures thereof.

31. A composition according to claim 26 wherein said compound is a protease inhibitor.

32. A composition according to claim 31 wherein said compound is a serine protease inhibitor.

33. A composition according to claim 32 wherein said compound is a thrombin and/or trypsin and/or tryptase inhibitor or an inhibitor of the serine proteases naturally occurring in the skin which activate PAR-2.

34. A composition according to claim 33 wherein said compound contains a d-phenylalanine-proline-arginine motif.

35. A composition according to claim 33 wherein said compound is (S)-N-Methyl-D-phenylalanyl-N-[4-[(aminoiminomethyl)amino]-1-(2-benzothiazolylcarbonyl)butyl]-L-prolinamide.

36. A composition according to claim 31 wherein said compound is a naturally-derived product which affects the PAR-2 pathway.

37. A composition according to claim 36 wherein said compound is derived from one or more of the botanical families leguminosae, solanaceae, gramineae and cucurbitaceae.

38. A composition according to claim 37 wherein said compound is derived from legumes.

39. A composition according to claim 38 wherein said compound is derived from soy, lima and/or black beans.

40. A composition according to claim 39 wherein said compound is selected from a group consisting of:

undenatured soybean extract, limabean extract, blackbean extract or mixtures thereof.

41. A composition according to claim 40 wherein said compound is selected from the group consisting of soybean milk, limabean milk, blackbean milk, soybean extract, limabean extract, blackbean extract, soybean paste, limabean paste and blackbean paste and mixtures thereof.

42. A composition according to claim 40 wherein said compound is a fraction of soybean milk, soybean extract, soybean paste, limabean milk, limabean extract, limabean paste, blackbean milk, blackbean extract, blackbean paste and mixtures thereof.

43. A composition according to claim 26 wherein said compound is a melanosome transfer inhibitor.

44. A composition according to claim 26 wherein said compound activates the PAR-2 pathway.

45. A composition according to claim 44 wherein said compound is a PAR-2 agonist which binds to and activates PAR-2.

46. A composition according to claim 45 wherein said compound is selected from the group consisting of SLIGRL, SLIGKVD and derivatives of SLIGRL and SLIGKVD which bind to and activate PAR-2 and mixtures thereof.

47. A composition according to claim 26 wherein said compound is a protease which activates PAR-2.

48. A composition according to claim 47 wherein said compound is a serine protease which activates PAR-2.

49. A composition according to claim 48 wherein said compound is selected from the group consisting of trypsin, tryptase, thrombin and proteases naturally-occurring in the skin which activates PAR-2.

50. A composition according to claim 26 wherein said compound is a melanosome transfer enhancer.

51. A composition according to claim 26 wherein said PAR-2 affecting compound is present in an amount of from about 0.0001% to about 15% by weight/volume of said composition.

52. A composition according to claim 51 wherein said compound is present in an amount from about 0.001 to about 5% of said composition.

53. A composition according to claim 52 wherein said compound is present in an amount from about 0.005 to about 1% of said composition.

54. A composition according to claim 26 comprising bean milk in an amount of from about 1 to about 99% by weight.

55. A composition according to claim 26 comprising soybean trypsin inhibitor, limabean trypsin inhibitor or blackbean trypsin inhibitor in an amount of from about 0.01 to about 20% by weight.

56. A method according to claim 1 wherein said composition is applied twice daily for at least eight weeks.

57. A method according to claim 56 wherein said composition is applied at a relatively high dosage for at least about four to about ten weeks and then applied at a relatively lower dosage on a continuous basis to maintain skin lightening effect.

58. A method according to claim 1 wherein said composition is administered orally.

59. A method according to claim 1 wherein said composition is administered parenterally.

60. A cosmetic composition according to claim 26 comprising said pigmentation-affecting compound and a cosmetically-acceptable vehicle.

61. A composition according to claim 60 wherein said composition further comprises additional depigmenting agents.

62. A composition according to claim 61 wherein said composition further comprises tyrosinase inhibitors.

63. A composition according to claim 26 wherein said composition further comprises liposomes.

64. A composition according to claim 63 wherein said composition comprises glycerol dilaurate, cholesterol, polyoxyethylene-10-stearyl ether and polyoxyethylene-9-lauryl ether.

65. A composition according to claim 26 wherein said composition further comprises anti-oxidants.

66. A composition according to claim 26 wherein said composition further comprises a sunscreen.

67. A composition according to claim 41 wherein said composition comprises from about 1 to about 99% bean milk, from about 0.1 to about 20% emulsifier and a preservative in an effective amount.

68. A composition according to claim 26 wherein said composition further comprises a compound selected from the group consisting of: anti-oxidants, sunscreens, moisturizers, bleaching agents, depigmentation agents, surfactants, foaming agents, conditioners, humectants, fragrances, viscosifiers, buffering agents, preservatives and a mixture thereof.

FIG. 1A

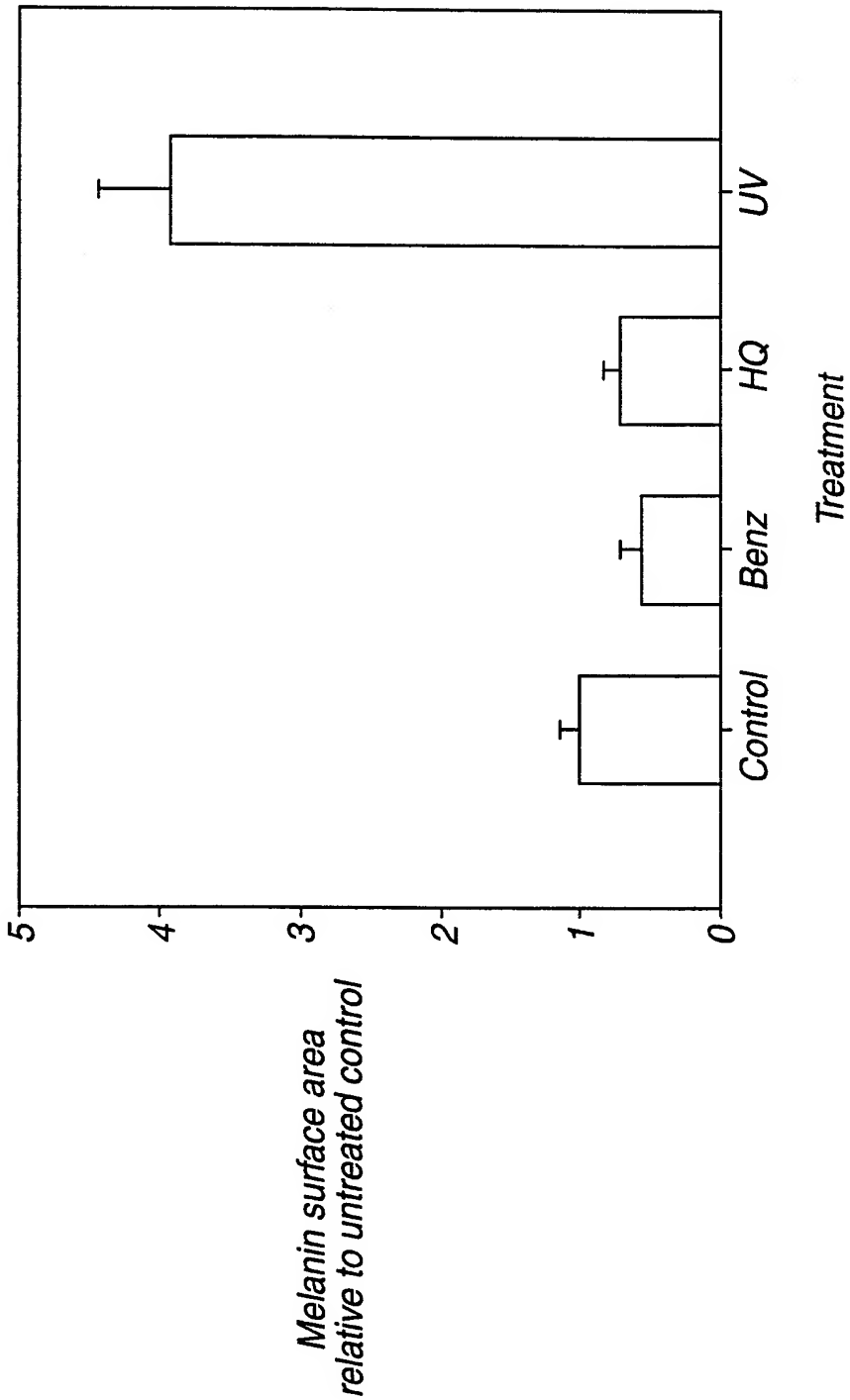

Melanin surface area
relative to untreated control
0
1
2
3
4
5
Control
Benz
HQ
UV
Treatment

FIG. 1B

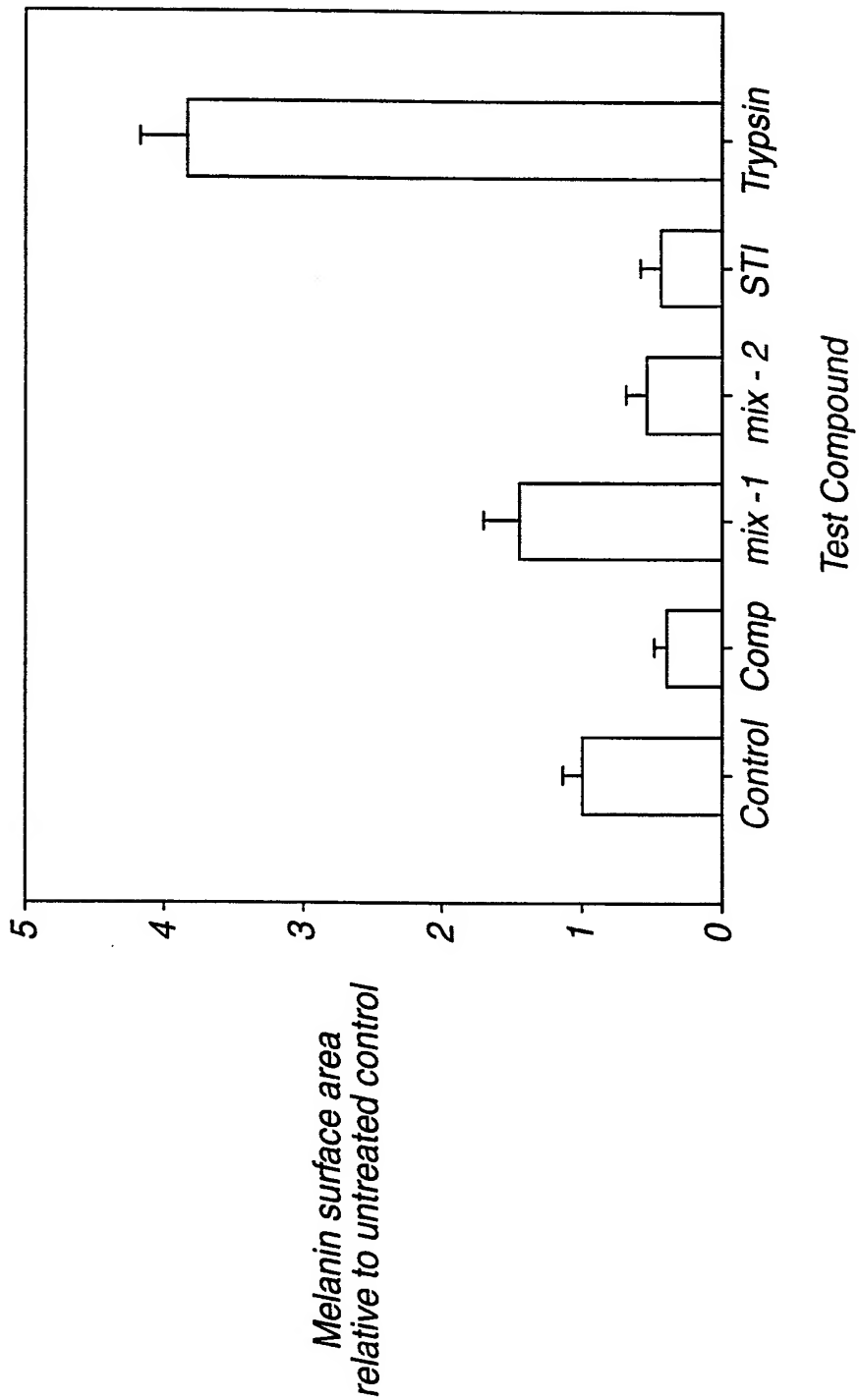

Melanin surface area
relative to untreated control
0
1
2
3
4
5
Control
Comp
mix -1
mix - 2
STI
Trypsin
Test Compound

FIG. 2

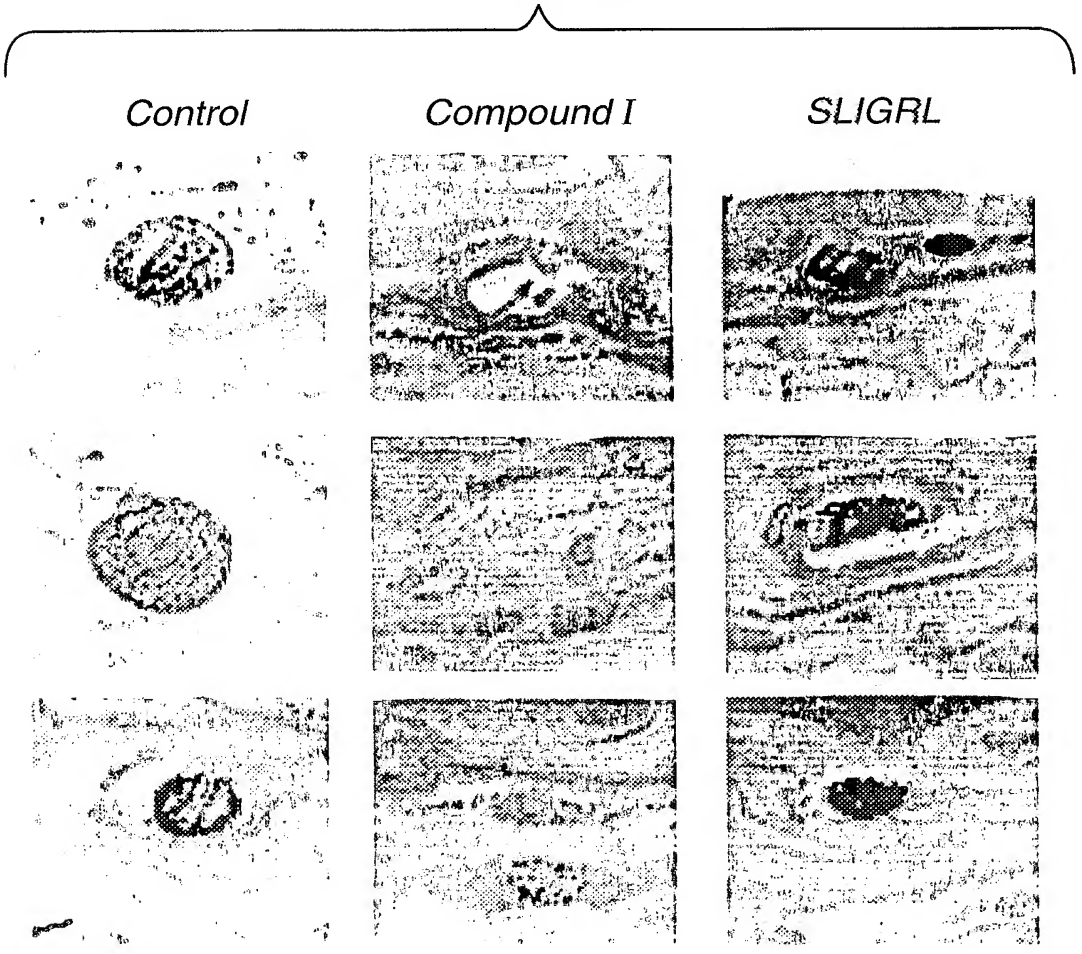


FIG. 3

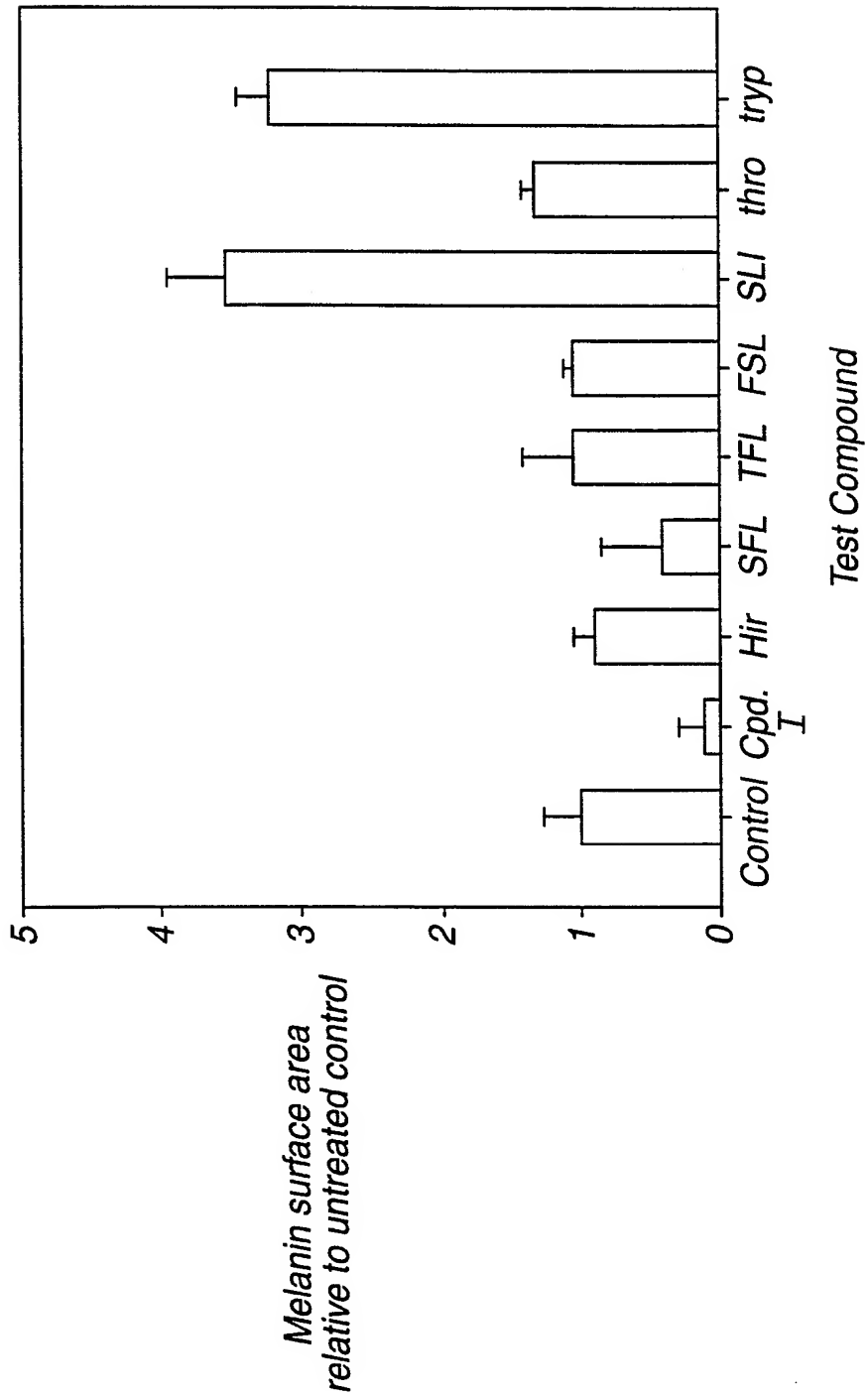

Melanin surface area relative to untreated control
0
1
2
3
4
5
Control
Cpd. I
Hir
SFL
TFL
FSL
SLI
thro
tryp
Test Compound

FIG. 4A

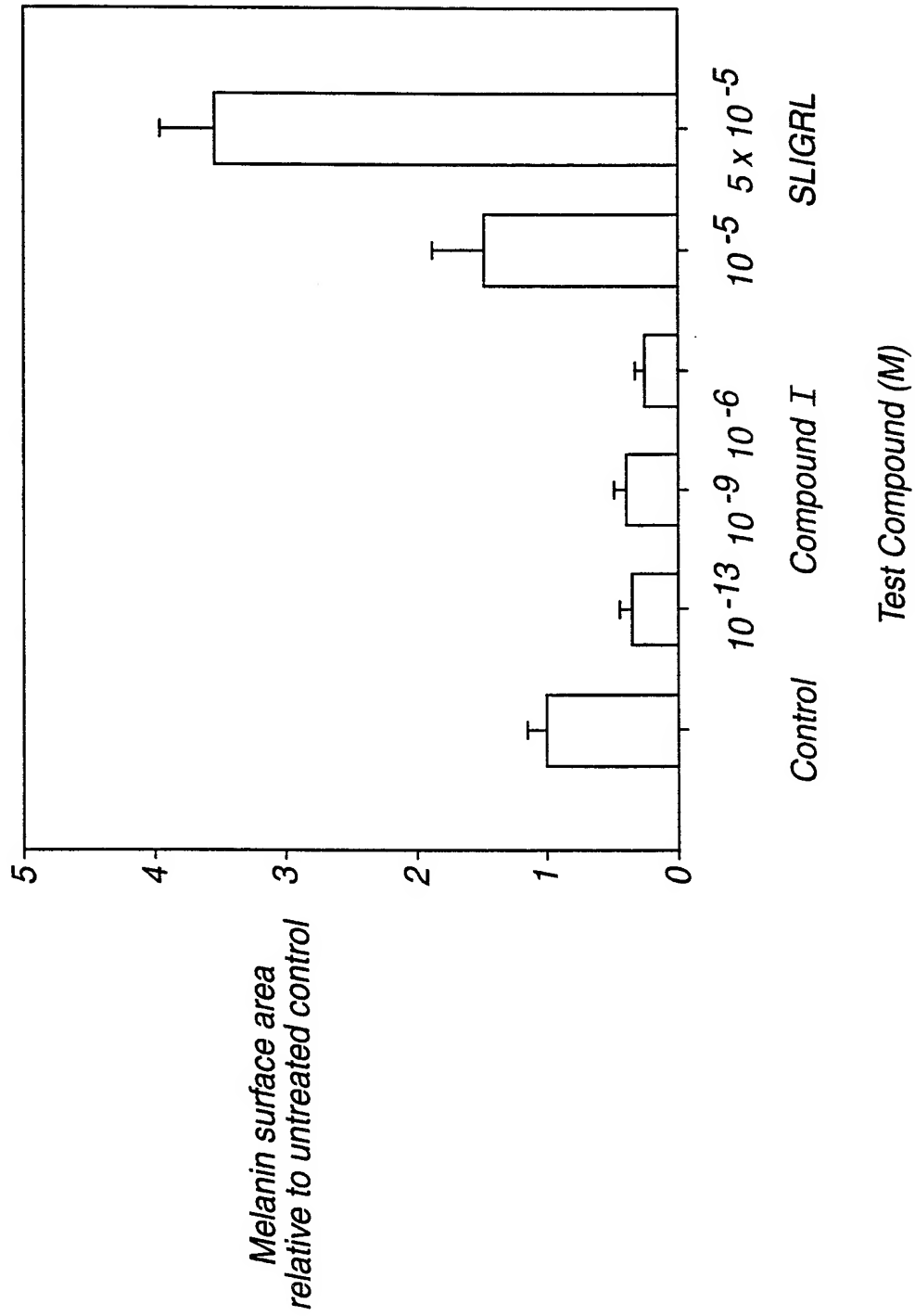

Melanin surface area relative to untreated control
0
1
2
3
4
5
Control
10 -13
10 -9
10 -6
10 -5
5 x 10 -5
Compound I
SLIGRL
Test Compound (M)

FIG. 4B

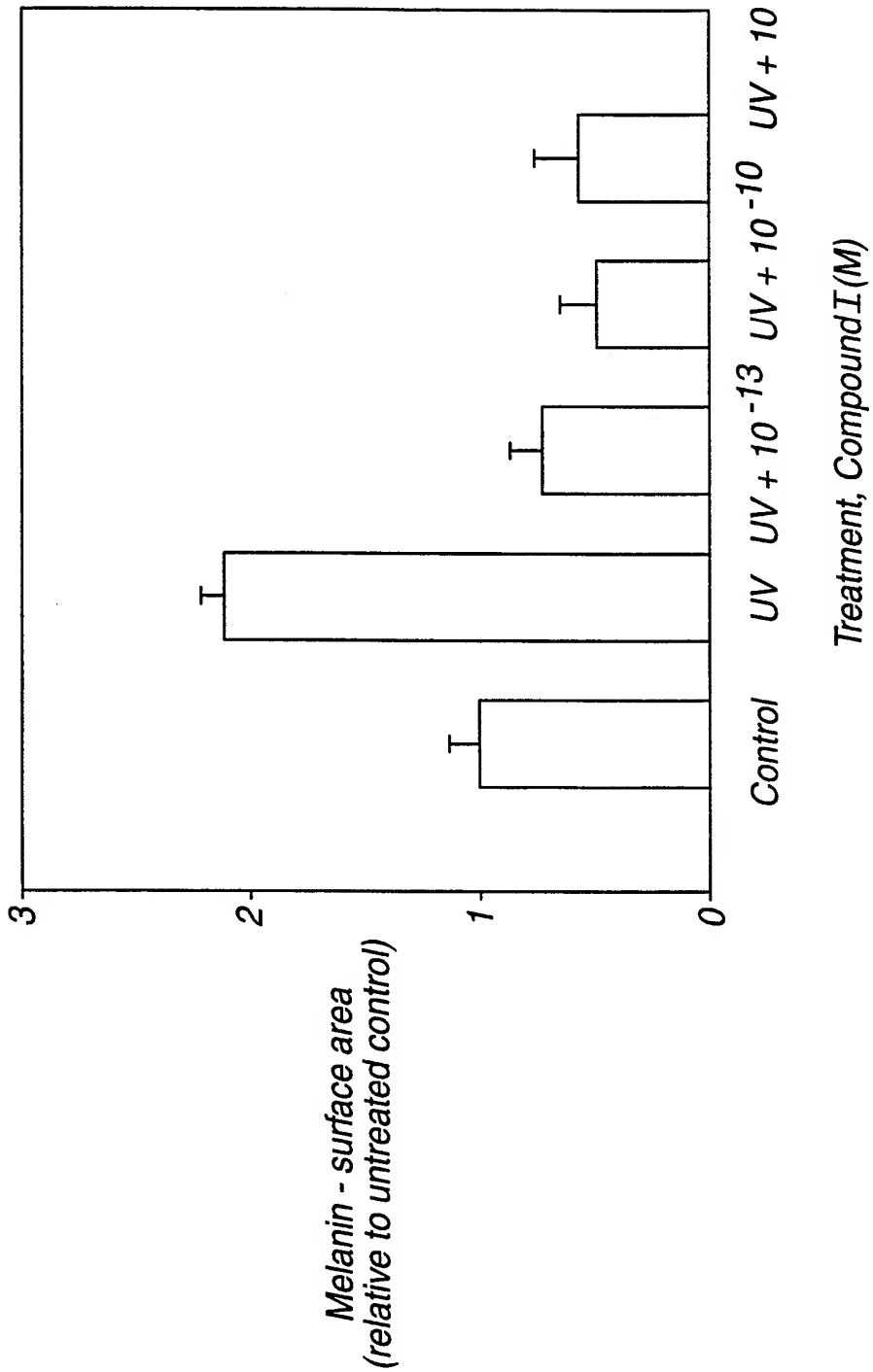

Melanin - surface area
(relative to untreated control)
0
1
2
3
Control
UV
UV + 10-13
UV + 10-10
UV + 10-7
Treatment, Compound I (M)

FIG. 5A

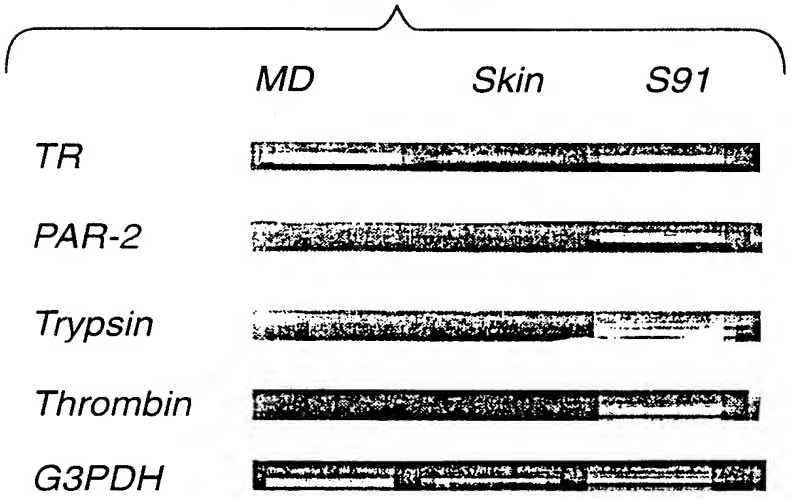


FIG. 5B

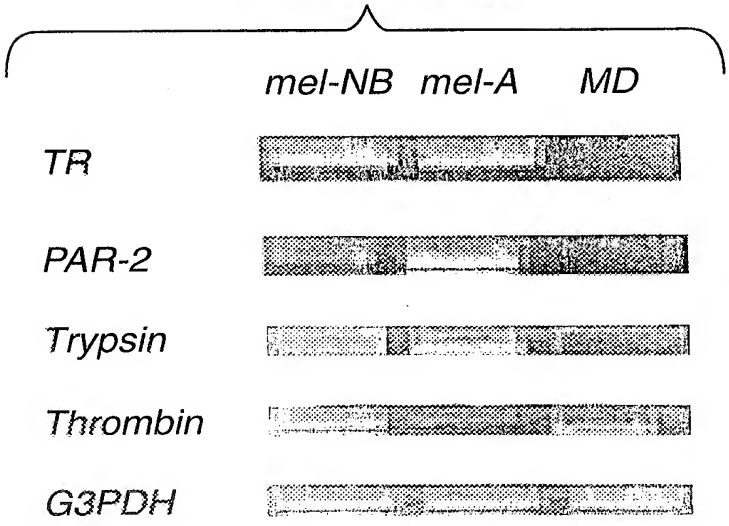


FIG. 6A

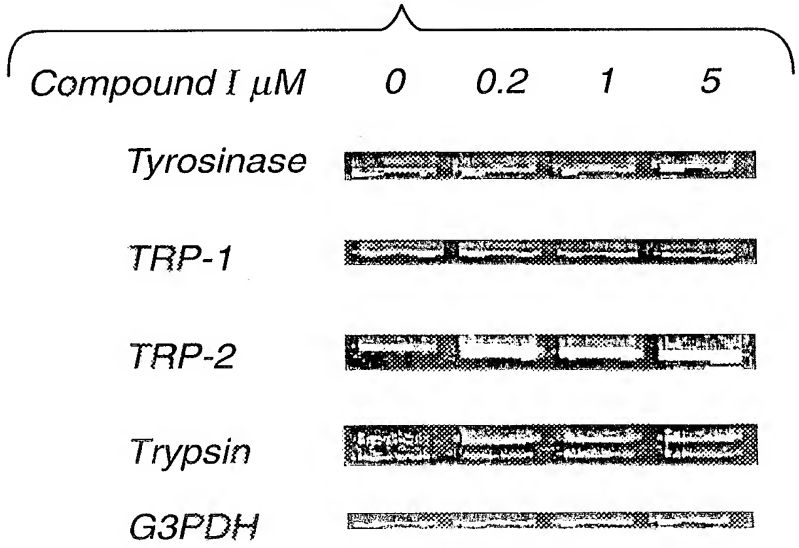


FIG. 6B

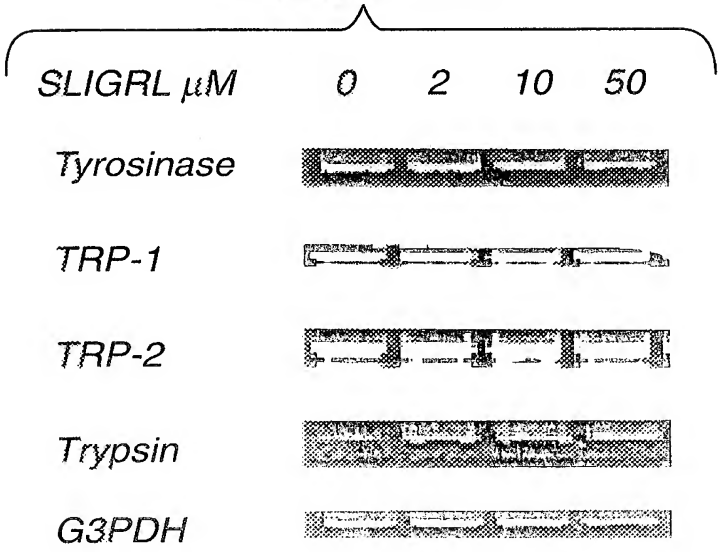


FIG. 7

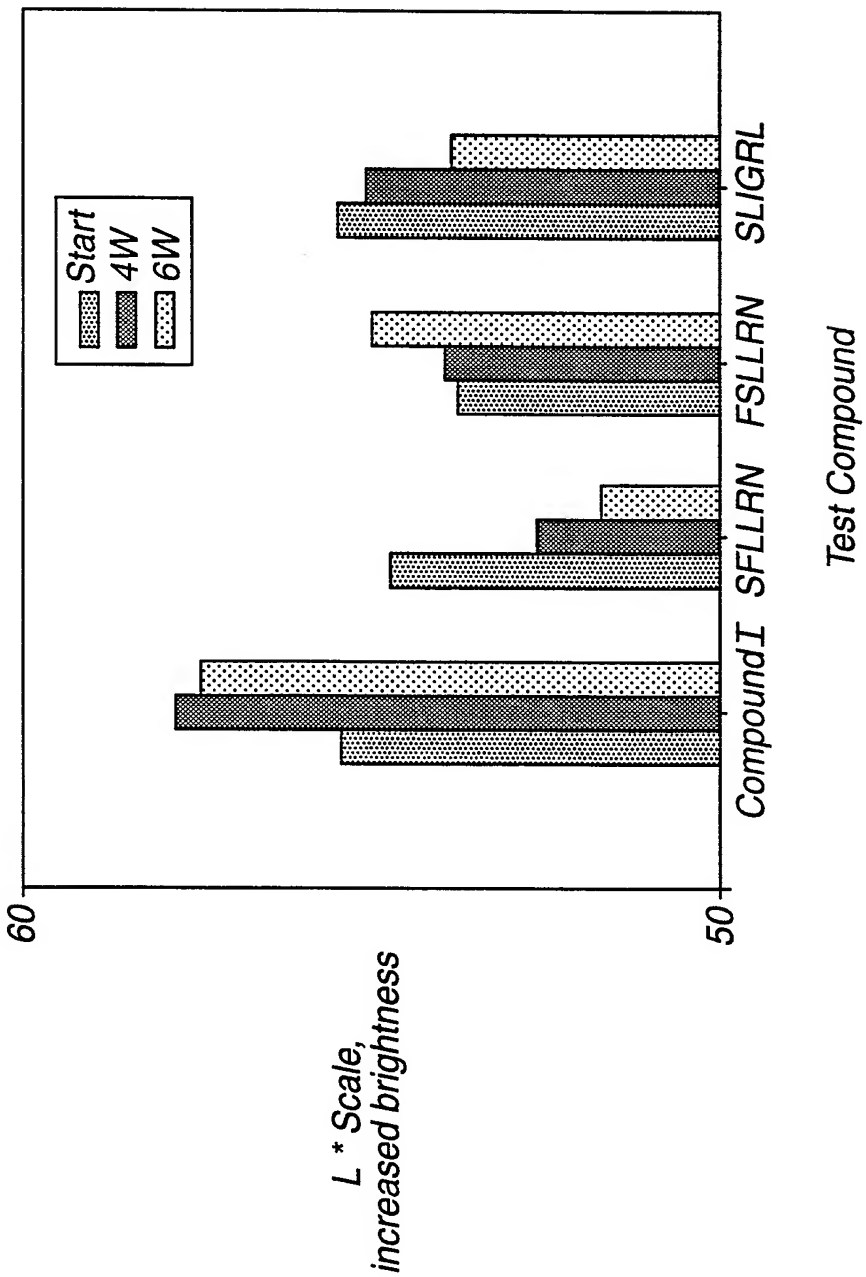

Start
4W
6W
60
50
L * Scale,
increased brightness
CompoundI
SFLLRN
FSLLRN
SLIGRL
Test Compound

FIG. 8

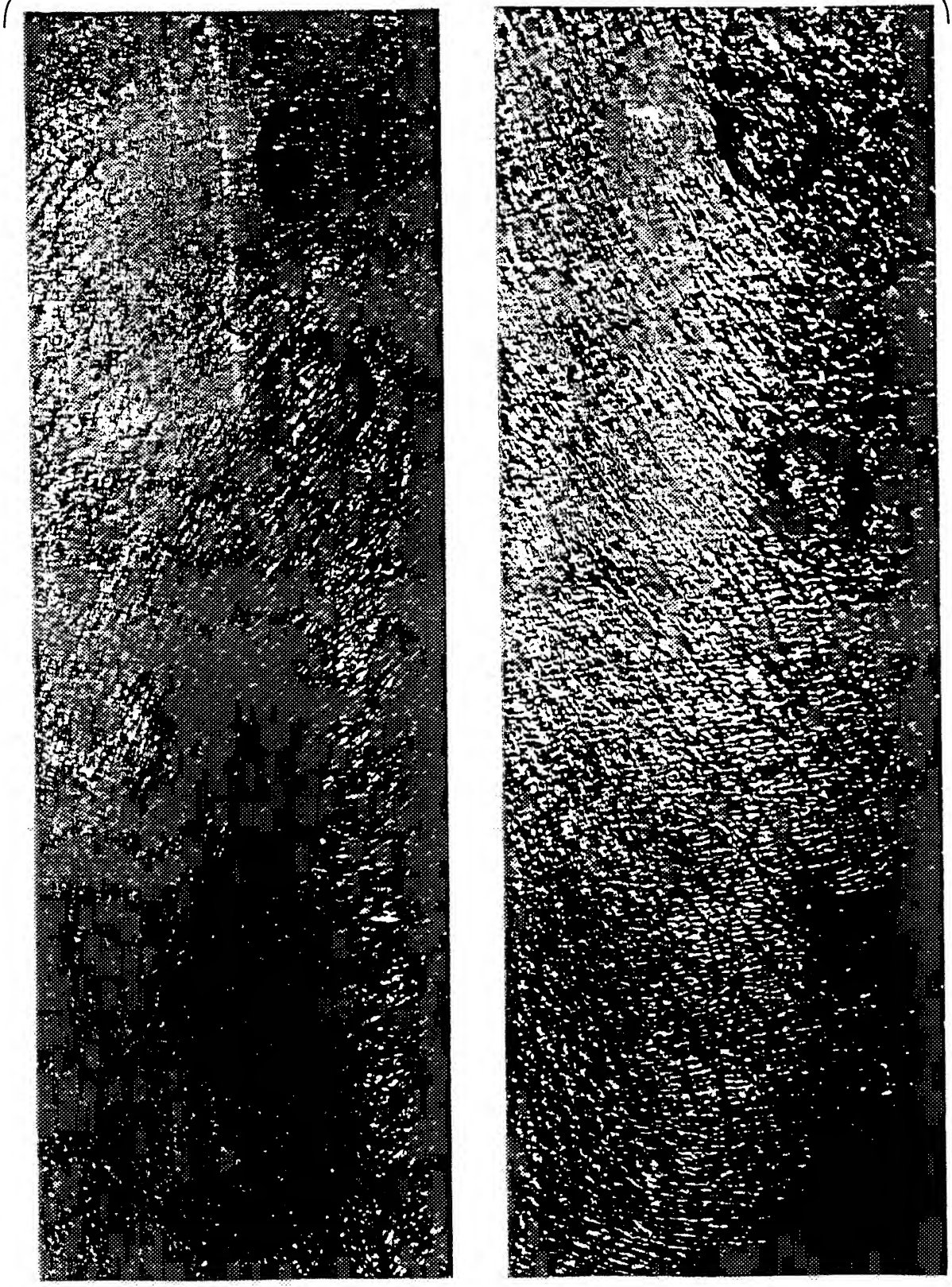


FIG. 9

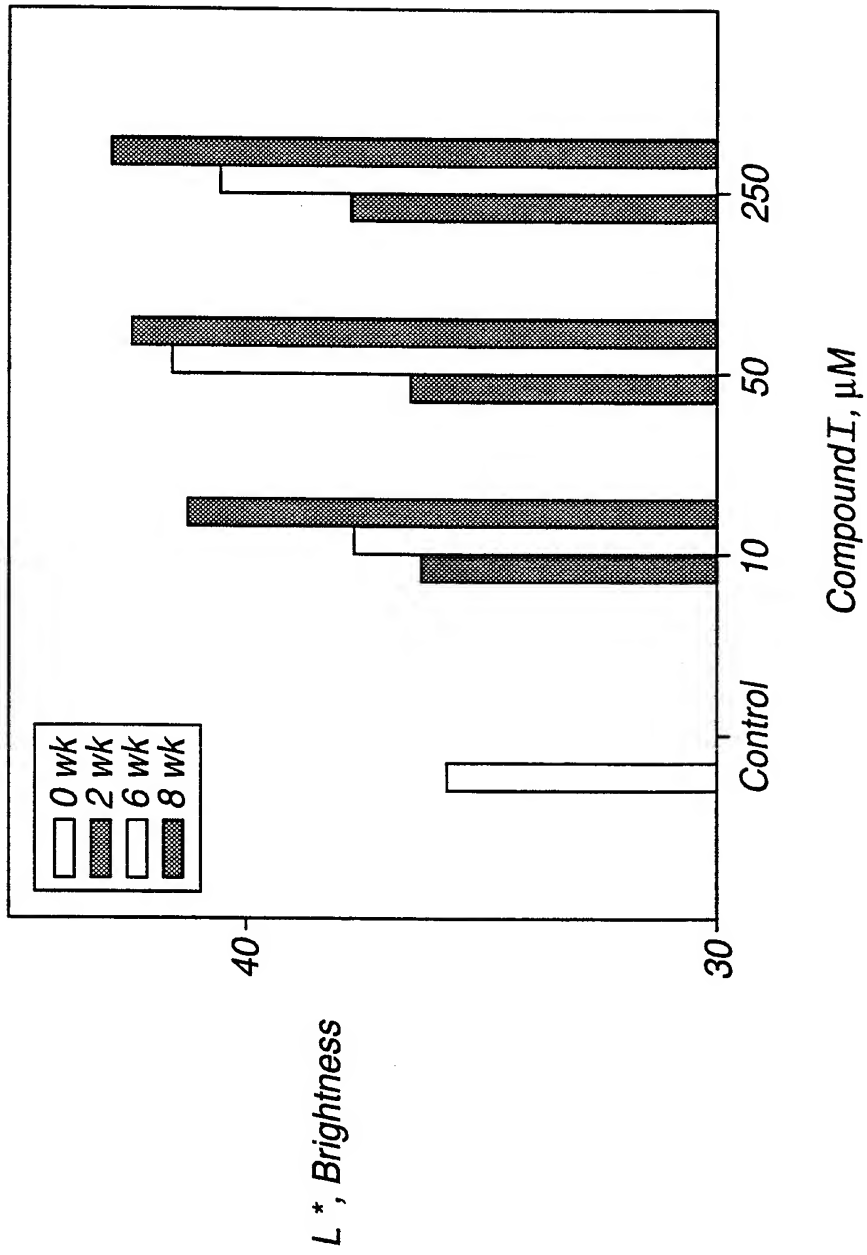

0 wk
2 wk
6 wk
8 wk
40
30
L *, Brightness
Control
10
50
250
Compound I, μM

FIG. 10D

FIG. 10C

FIG. 10B

FIG. 10A

FIG. 11B

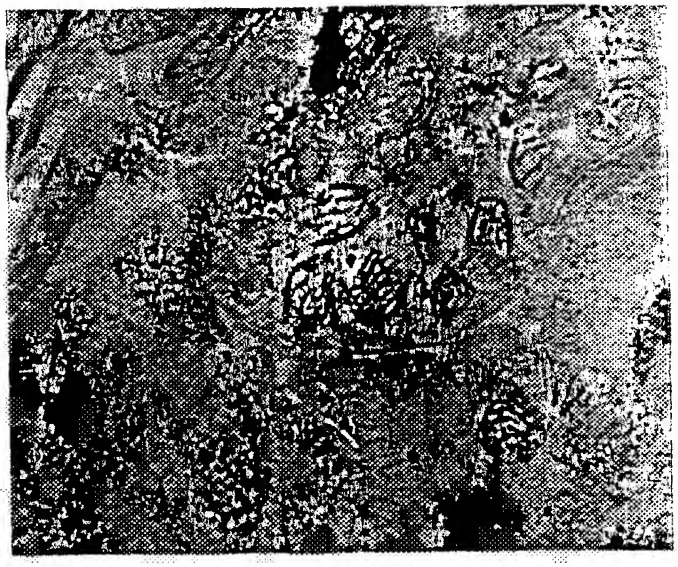


FIG. 11A

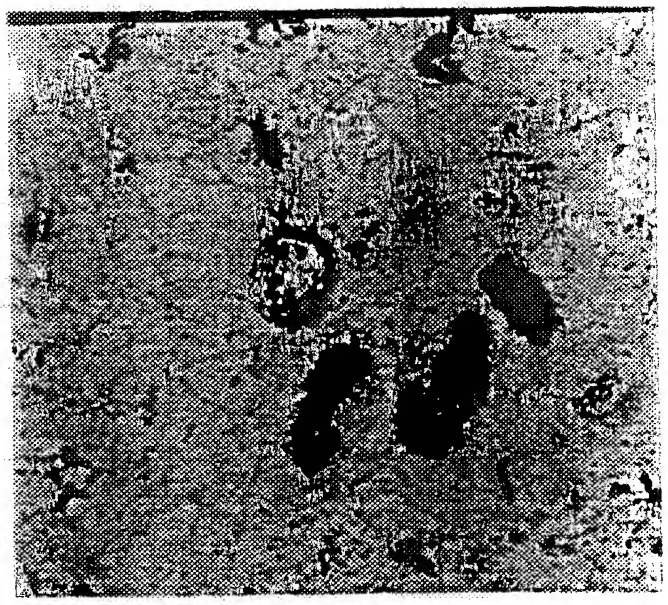


FIG. 11C

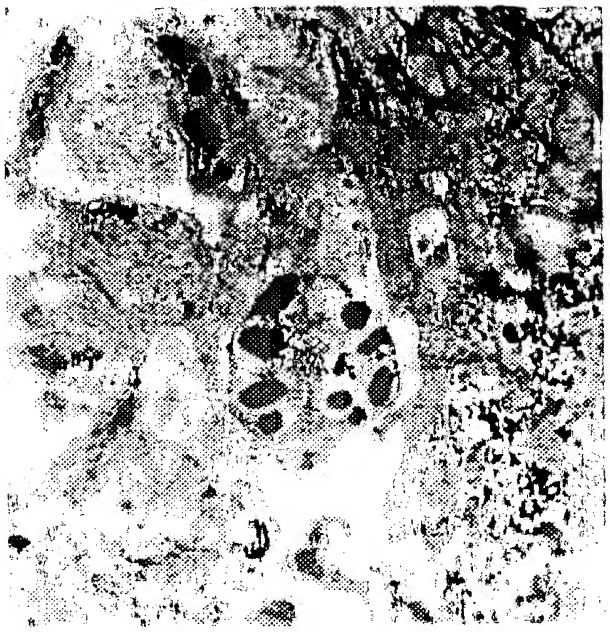


FIG. 11F

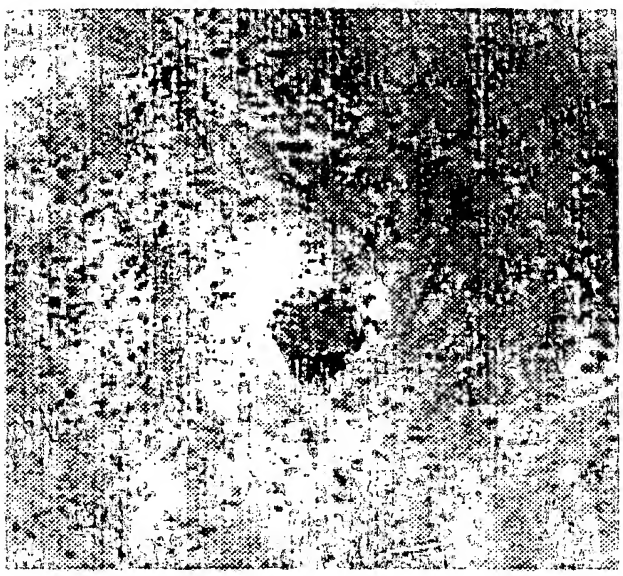


FIG. 11E

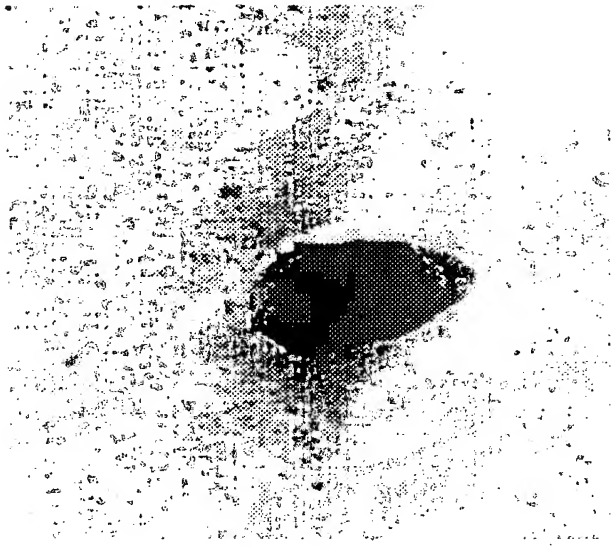


FIG. 11D

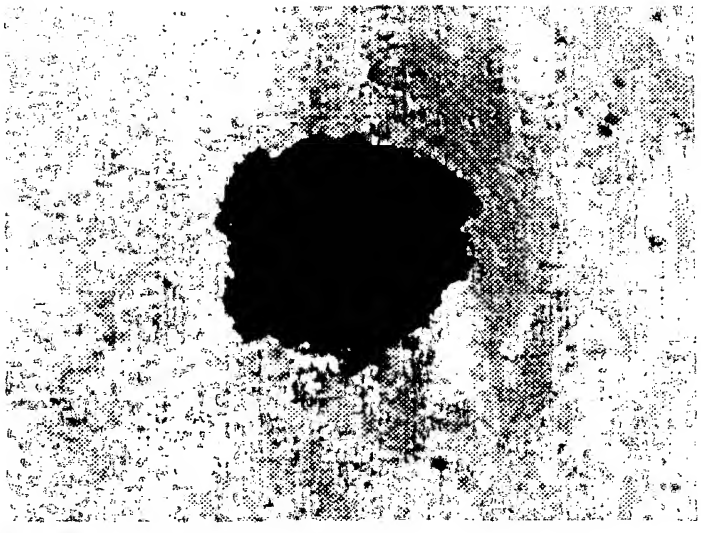


FIG. 11H

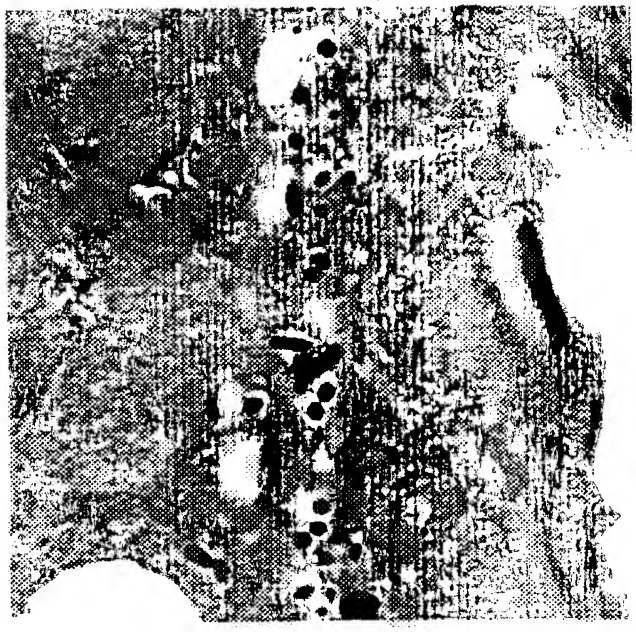


FIG. 11G

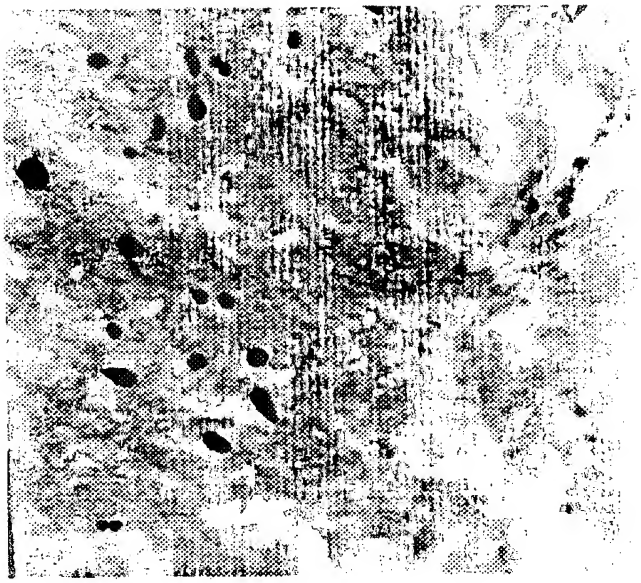


FIG. 12A

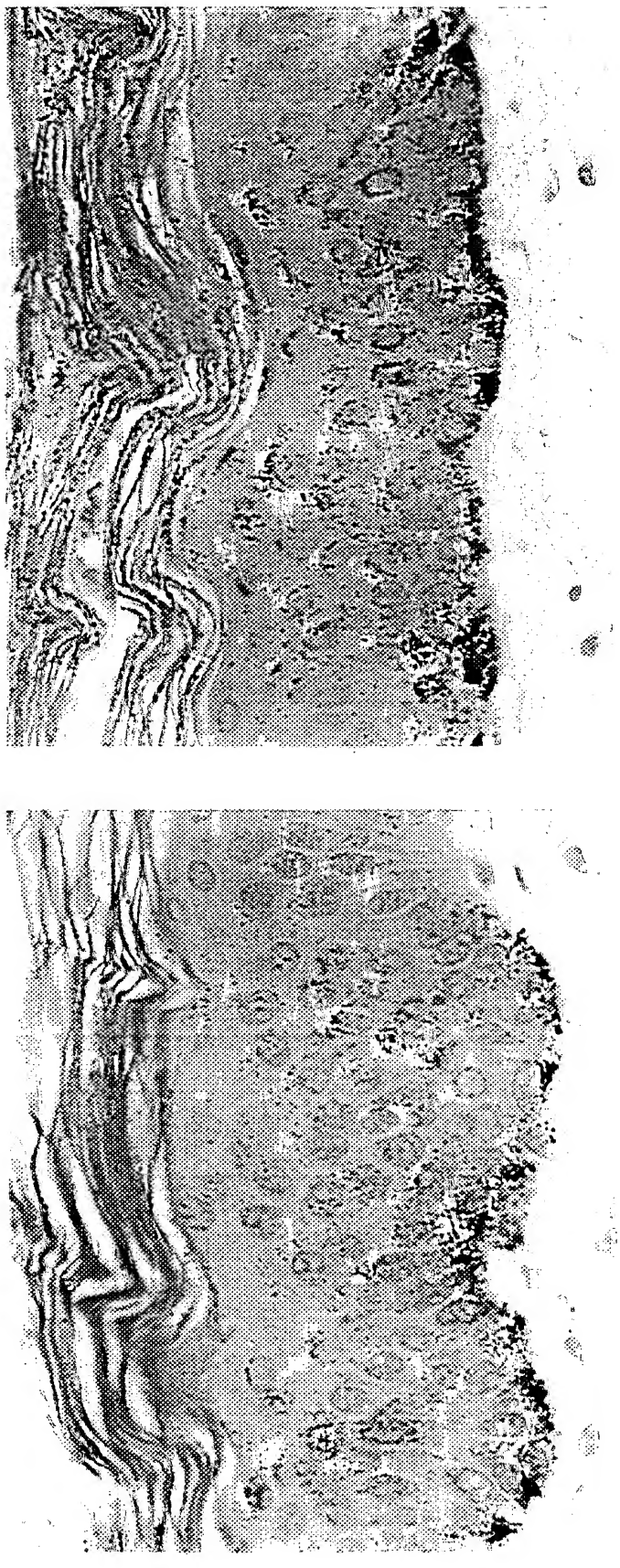
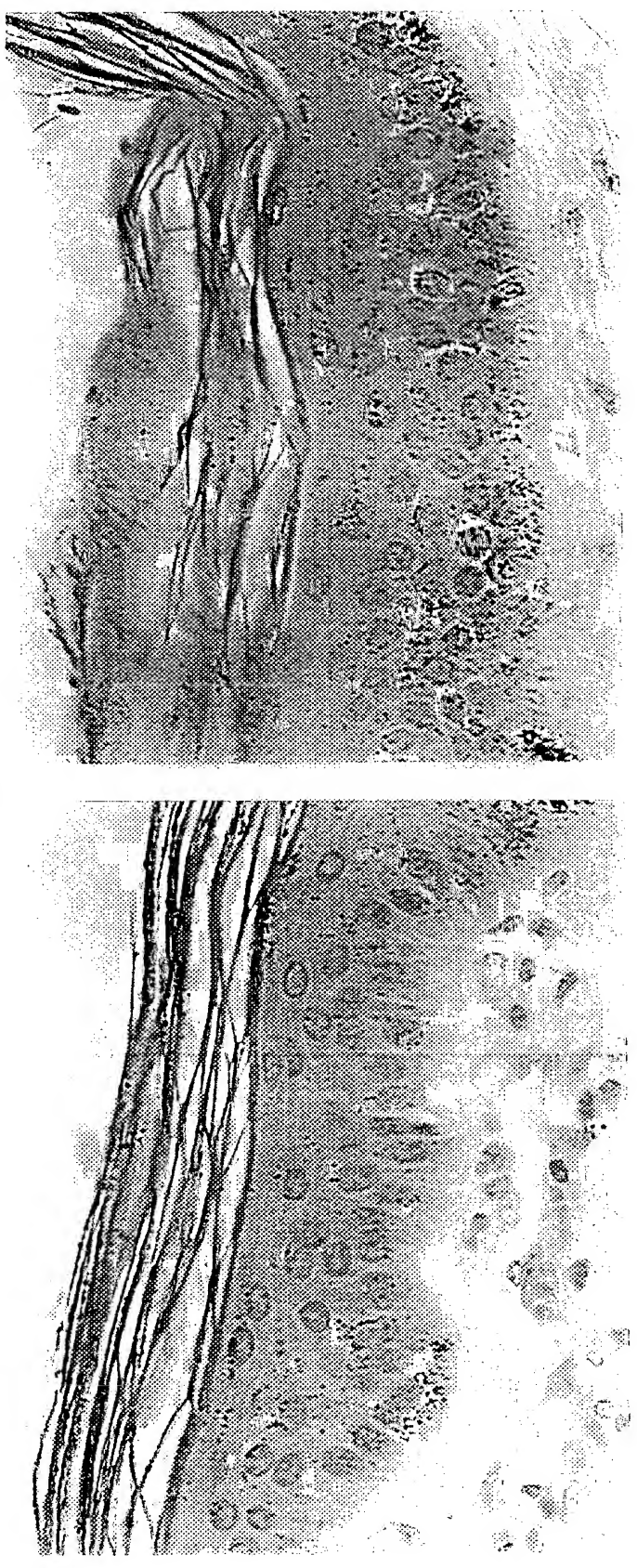
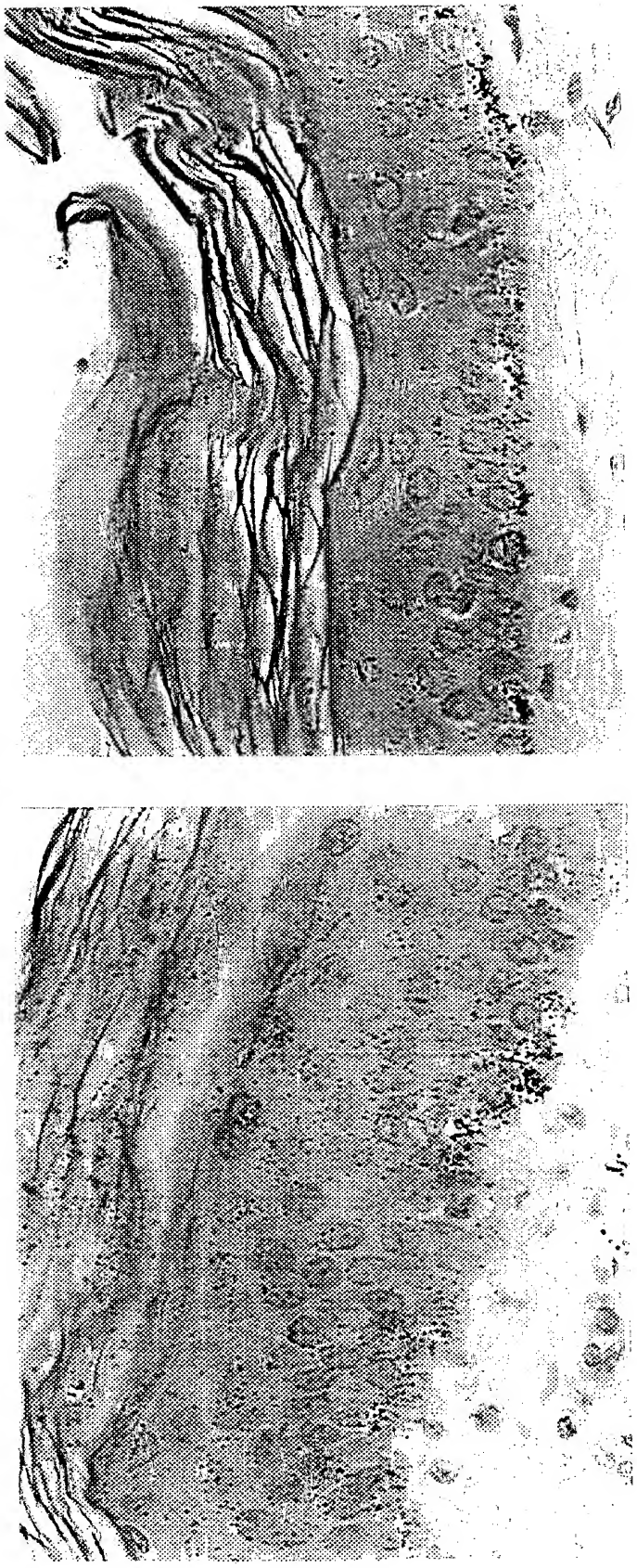


FIG. 12B



Compound I

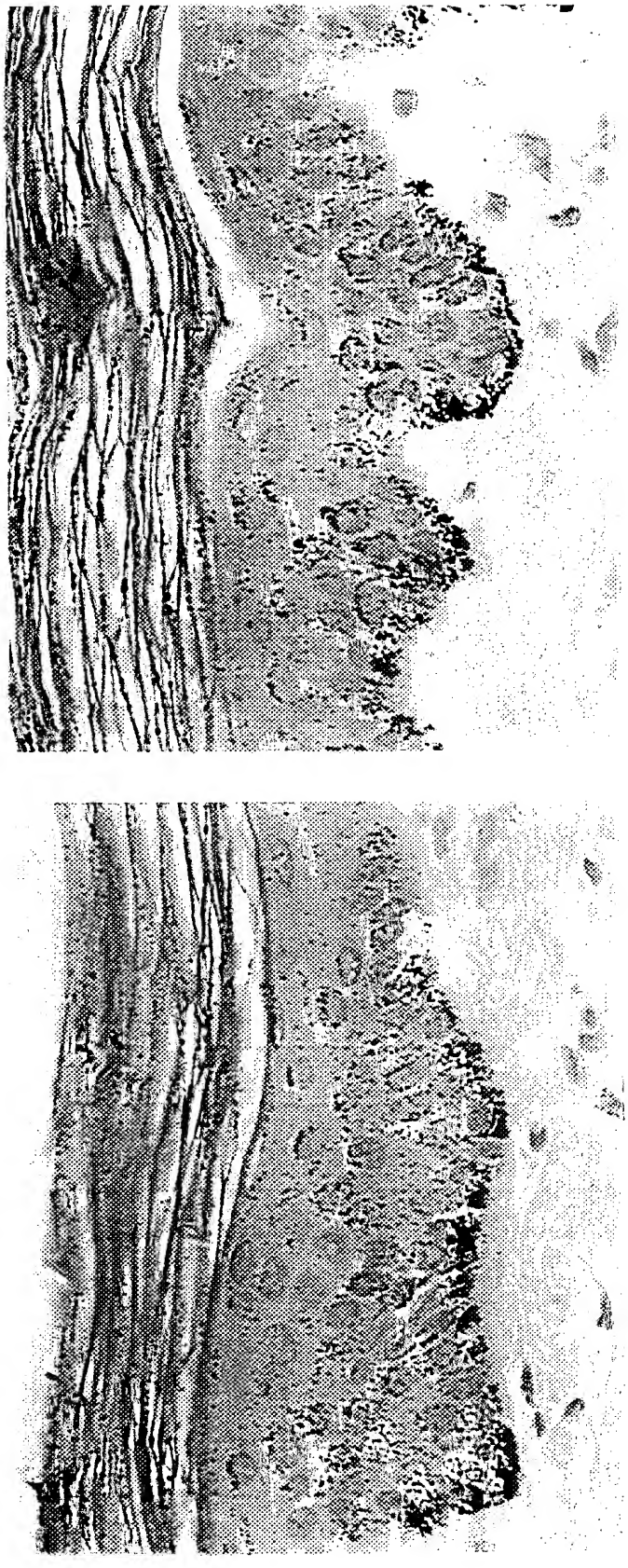
FIG. 12C



+1 Week

FIG. 12D

FIG. 12E



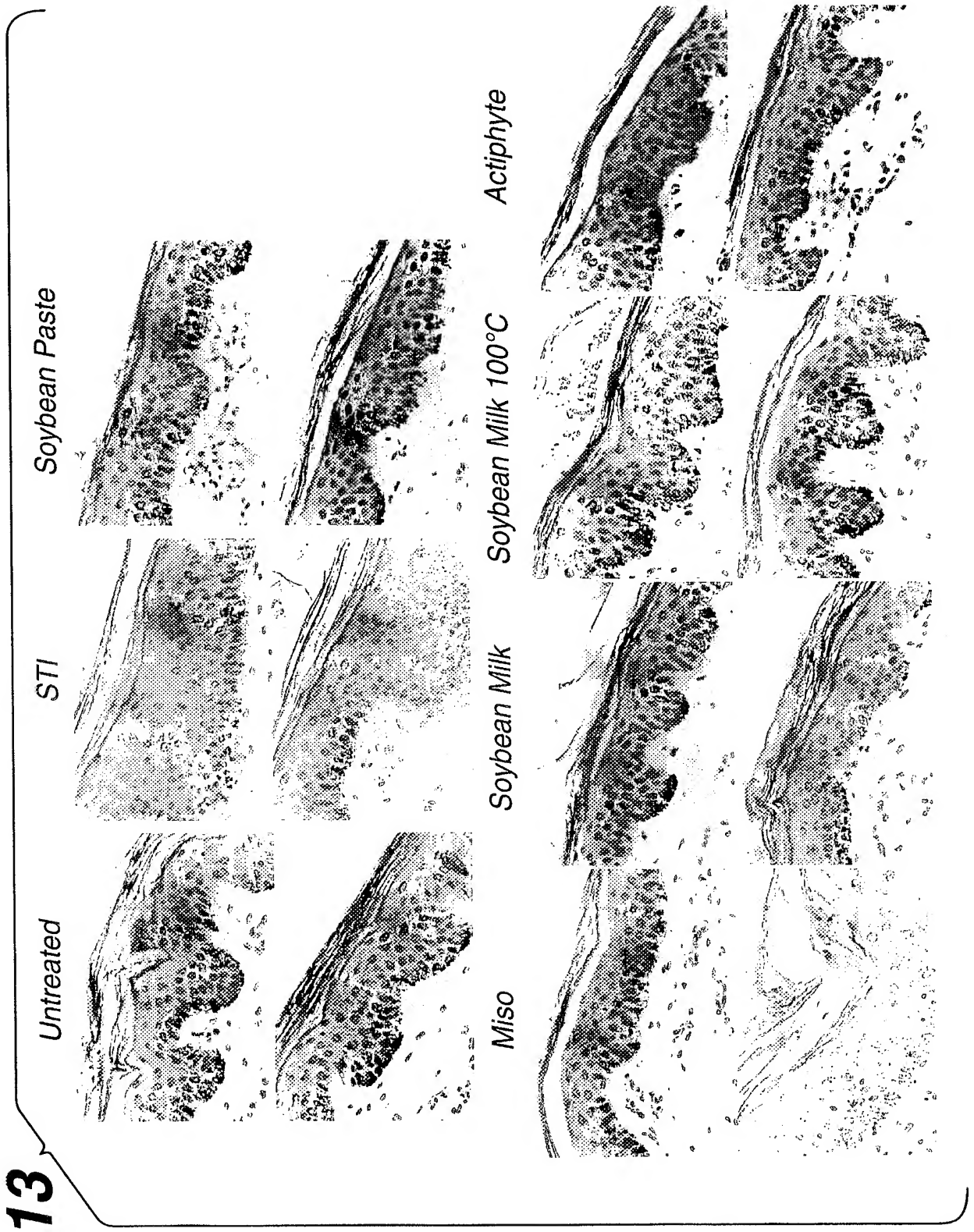

Untreated
STI
Soybean Paste
Miso
Soybean Milk
Soybean Milk 100°C
Actiphyte

FIG. 13

FIG. 14